MEDICAL LIBRARY

JAN 15 1947

CANCER RESEARCH

VOLUME 7
NUMBER 1
JANUARY, 1947

A MONTHLY JOURNAL
OF ARTICLES AND ABSTRACTS
REPORTING CANCER RESEARCH

CONTENTS

A. R. GOPAL-AYENGAR and E. V. COWDRY. Desoxyribose Nucleic Acid from Isolated Chromosome Threads in Experimental Methylcholanthrene Carcinogenesis in Mice 1

CHRISTOPHER CARRUTHERS and V. SUNTZEFF. Succinic Dehydrogenase and Cytochrome Oxidase in Epidermal Carcinogenesis Induced by Methylcholanthrene in Mice 9

EDWARD W. SHRIGLEY, HARRY S. N. GREENE, and F. DURAN-REYNALS. Growth of Avian Tumors Other Than the Rous Sarcoma in the Anterior Chamber of the Guinea Pig Eye 15

F. DURAN-REYNALS and J. W. KING. Reciprocal Effects of Natural Immune Bodies from Chickens and Ducks on Variants of a Sarcoma Virus 21

PAUL N. HARRIS. On the Production of Sarcoma with Wheat Germ Oil 26

PAUL N. HARRIS. Production of Sarcoma in Rats with Light Green SF 35

A.A.A.S. GIBSON ISLAND RESEARCH CONFERENCE ON CANCER, 1946. Abstracts of Papers Presented 37

ABSTRACTS 53-63
Reports of Research 53-57
Clinical and Pathological Reports 57-63

BOOK REVIEWS 64

THE OFFICIAL ORGAN OF THE
AMERICAN ASSOCIATION FOR CANCER RESEARCH, INC.

CANCER RESEARCH

This journal is sponsored by the American Association for Cancer Research, Inc., The Anna Fuller Fund, The Donner Foundation, Incorporated, Cancer Research Division, and The Jane Coffin Childs Memorial Fund for Medical Research.

Advisory Board

MILDRED W. S. SCHRAM, *Chairman*

S. BAYNE-JONES
M. J. SHEAR
JAMES B. MURPHY
GEORGE M. SMITH

Editorial Committee

JAMES B. MURPHY, *Chairman*
S. BAYNE-JONES, *Editor*
E. W. SHRIGLEY, *Editor, Abstracts Section*

JOHN J. BITTNER
ALEXANDER BRUNSCHWIG
E. V. COWDRY
LOUIS I. DUBLIN
GIOACCHINO FAILLA
LOUIS F. FIESER
JACOB FURTH
WILLIAM U. GARDNER
JESSE P. GREENSTEIN
FRANCES L. HAVEN
BALDUIN LUCKÉ
E. C. MACDOWELL
G. BURROUGHS MIDER
EDGAR G. MILLER, JR.
JOHN J. MORTON
EDITH H. QUIMBY
MURRAY J. SHEAR
HAROLD L. STEWART
GRAY H. TWOMBLY
SHIELDS WARREN

Abstractors

C. AUGER
E. B. BARBER
W. A. BARNES
S. BAYNE-JONES
M. BELKIN
E. BOYLAND
R. BRIGGS
W. J. BURDETTE
A. CLAUDE
A. CORNELL
H. J. CREECH
Z. DISCHE
L. J. DUNHAM
W. E. GYE
A. HADDOW
J. B. HAMILTON
F. L. HAVEN
J. HEIMAN
I. HIEGER
G. H. HOGEBOOM
M. E. HOWARD
R. A. HUSEBY
R. JAHIEL
R. N. JONES
H. S. KAPLAN
E. L. KENNAWAY
J. G. KIDD
A. KIRSCHBAUM
M. LEDERMAN
M. L. LEVIN
V. J. LONGO
R. J. LUDFORD
J. L. MELNICK
A. MELTZER
C. R. NOBACK
M. H. PESKIN
C. A. PFEIFFER
K. R. PORTER
L. W. PRICE
E. H. QUIMBY
E. C. RICHARDSON
D. SHEMIN
R. E. SNYDER
E. E. SPROUL
M. TAFFEL
C. WARREN
G. W. WOOLLEY

Published by The Donner Foundation, Incorporated, Cancer Research Division.
Publication Office, 125 Temple Street, New Haven 10, Connecticut

The annual subscription rates for one volume are: To members of the American Association for Cancer Research, Inc., $5.00; to others and to libraries, institutions, and organizations, $7.00. Business communications, remittances, and subscriptions should be addressed to Theodore P. Eberhard, Business Manager, 2136 Land Title Building, Philadelphia 10, Pa.

No responsibility is accepted by the Committee, by the Board, or by the Publishers of *Cancer Research* for opinions expressed by contributors.

Entered as second class matter December 16, 1946, at the Post Office at New Haven, Conn., under the Act of March 3, 1879.

Copyright, 1946, by The Donner Foundation, Incorporated, Cancer Research Division.

SEE INSIDE BACK COVER FOR INFORMATION FOR CONTRIBUTORS

CANCER RESEARCH
A MONTHLY JOURNAL OF ARTICLES AND ABSTRACTS REPORTING CANCER RESEARCH
VOLUME 7 JANUARY, 1947 NUMBER 1

Desoxyribose Nucleic Acid from Isolated Chromosome Threads in Experimental Epidermal Methylcholanthrene Carcinogenesis in Mice*

A. R. Gopal-Ayengar, M.Sc., M.A., Ph.D., and E. V. Cowdry, Ph.D.

(*From the Barnard Free Skin and Cancer Hospital, St. Louis 3, and the Department of Anatomy, Washington University School of Medicine, St. Louis 10, Missouri*)

(Received for publication August 15, 1946)

INTRODUCTION

In the major cancer research project conducted at this hospital, cytological, biochemical and biophysical changes that supervene in the epidermis of mice as a result of applications of methylcholanthrene have been followed. This approach to the problem of induction of malignancy has resulted in the discovery of distinct cellular and intercellular transformations leading to an early establishment of a new chemical equilibrium in the altered epidermis, which is maintained, substantially without further modifications, throughout the "latent period" of carcinogenesis. The steps by which these transformations are effected have been integrated and embodied in reports published by Cowdry (12-14).

The biological importance of nucleic acids in growth and development has recently been reviewed by Davidson and Waymouth (20) and by Davidson (19). There is increasing recognition of a causal connection between the processes connected with growth and the spatial distribution of nucleic acids in the cell. Thus disturbances in the cellular nucleic acids accompanying neoplasia have been noted by Caspersson and Santesson (7), Koller (24) and Stowell (33, 35). Stowell and Cooper (34) measured the relative amounts of Feulgen stained thymonucleic acid in normal epidermis, hyperplastic epidermis and epidermoid carcinoma with a microphotometric apparatus. They detected in general a fall in the thymonucleic acid content per unit volume of hyperplastic epidermis, and a rise in the carcinomas as compared with the normal epidermis. Carruthers and Suntzeff (6) have recently made quantitative determinations of desoxyribose nucleic acid in epidermal cells during carcinogenesis. They observed a progressive decrease in the amount of this substance from normal to malignant stages through the intermediate hyperplasia. The observations of these last investigators are in harmony with their earlier findings on the trend of biochemical changes in epidermal carcinogenesis. Our objectives have been (a) to isolate chromosomes from the epidermal and carcinomatous cells and collect them as free as possible from other cellular constituents; (b) to determine quantitatively desoxyribose nucleic acid in these chromosomes during epidermal carcinogenesis.

MATERIAL AND METHODS

Swiss mice were treated with methylcholanthrene, as already described by Cowdry (12). Epidermis was separated from the underlying dermis at room temperature using the method devised by Suntzeff and Carruthers (36), which involves stretching the skin tightly over a board covered with transparent plastic and scraping the epidermis off the dermis. This technic is a distinct improvement over the one adopted earlier by Baumberger, Suntzeff and Cowdry (1) in that while the latter method employed a temperature of 50° C., the critical temperature for biological activities, for softening the collagenic fibers and thus to separate the epidermal layers from the corium, the new procedure is carried out at room temperature, thus eliminating the attendent dangers of water loss and deleterious effects on the cells.

The separated epidermis was immediately dropped

* Aided by grants from the National Cancer Institute and an anonymous donor.

into vials containing physiological saline surrounded by freezing mixtures or dry ice. In the same manner solid pieces of transplanted squamous cell carcinomas, free from visibly necrotic areas, were dropped into similar containers. The lines of transplantable skin carcinomas of mice, induced by methylcholanthrene, employed by us were started nearly five years ago by Cooper, Firminger and Reller (11), and to date have been carried through 40 passages. Healthy and non-necrotic tumors, kindly donated by Drs. Cooper and Suntzeff, were cut up into small pieces, approximately 2 mm. in size, and were introduced subcutaneously into mice 6 weeks old. Some mice of the same age were also injected subcutaneously with fine fragments of the tumor suspended in physiological saline. The rate of growth of the transplanted tumor in the mice was at least double that of the injected population judged by the time lapse between introduction of the material and expression of optimum growth. Best results were obtained with tumors harvested 2 weeks after implantation. Although there were individual variations between tumors of this age, the majority of them showed little necrosis or cellular degeneration. The frozen tissues showed no visible s.ructural alterations before isolation of chromosomes.

EXPERIMENTAL PROCEDURE

The technic of isolation of the chromosomes was that of Claude and Potter (9) and Mirsky and Pollister (25, 26) slightly modified. The basis of isolation was recognition of the fact that disruption by mechanical breakage of the nuclear and cell membranes releases the diverse components of the cellular system into the surrounding medium. As demonstrated by Bensley (2), Claude and Potter (9) and Mirsky and Pollister (25, 26) these components can be segregated, concentrated and purified by differentiial centrifugation. The final fraction readily lends itself to further study and chemical analysis. By using 1.5 per cent citric acid at about pH3 as employed by Dounce (22, 23) and Ziegler (38), the nuclei were loosened but at this low pH nucleoproteins were undoubtedly denatured. Moreover it was thought that the cells were subjected to a *milieu* antagonistic to normal cellular activities. Hence this experimental procedure was abandoned.

Following the technic of Claude and Potter (9), who used leukemic cells of the C58 strain of mice and leukemic Wistar rats, the epidermal tissues were ground with sand in a mortar before centrifugation, but the results were unsatisfactory because it was extremely difficult to eliminate the fine particles of sand from the final fractions. The method that was finally developed consisted of grinding the separated epidermis, or the fragments of tumor, in a coarse mortar at a temperature of 1° to 2° C. until most of the cells and nuclei were crushed. The crushed mass was frozen, suspended in physiological saline at a temperature of about 1° to 2° C., and further disintegrated by subjection to the action of a high speed Waring blendor which had been cooled in dry ice before use.

Although it was comparatively easy to disintegrate cancer cells by this method, both normal and hyperplastic epidermal cells offered a great deal of resistance and longer treatment, both in the mortar and blendor, was necessary to rupture them. Even so, some of the epidermal cells remained unbroken.

For a satisfactory separation, control of two variables was found to be of paramount importance, namely: the maintenance of a temperature between 0° and 5° C. and the regulation of pH from 7.2 to 7.4 throughout the course of the experiment. Under these conditions, the chromatin threads that are in suspension retain their distinctive fibrous appearance. The schedule of differential centrifugation was in all essential respects similar to that outlined by Claude. The instrument used was a model PR1 International Refrigerated Centrifuge adjusted to maintain a temperature of 0° C.

Two experimental errors are not completely eliminated: (a) Loss of some extremely fine chromatin strands near the limits of microscopical resolution because of their failure to sediment with the rest of the chromatin material, and (b) contamination of the chromatin threads by small amounts of cytoplasmic particles and mitochondria. Because these defects in the method are applicable more or less equally to all three types of tissues studied, they were not deemed serious enough to vitiate the results.

The chromosomes, thus isolated, are ready for microscopical examination and biochemical analysis. They are Feulgen-positive and give a distinct blue color when subjected to the diphenylamine reaction of Dische (21) as employed by Seibert (32), Dounce (23) and Carruthers and Suntzeff (6). In quantitative evaluation of the desoxyribose nucleic acid the Dische reaction was applied to isolated chromosomes dried *in vacuo*, and the percentage transmission of the characteristic blue color at 600 mμ was measured with a Coleman spectrophotometer. The standard curve was made by Dr. Carruthers from pure sodium desoxyribonucleate by plotting the concentration of this substance against the percentage transmission. The actual nucleic acid concentration was derived by multiplying the concentration of the sodium salt by 0.944.

OBSERVATIONS

Morphology.—Since most of the epidermal nuclei were in the resting stage and only a small proportion of them in various phases of mitoses, the bulk of the isolated chromosomes was evidently derived from the

resting nuclei. The position of the primary constriction is clearly recognizable in many of the chromosomes, especially in the stretched out condition. The centromeric regions appear as Feulgen-negative gaps between the long and short arms of the chromosomes (Figs. 1 to 3). The final purified sample appeared white and had a viscous consistency. Under dark field illumination the chromatin appeared as distinctly fibrous, rod-shaped or granular bodies of varying length but of more or less uniform diameter, the latter being usually from 0.5μ to 2μ. Many of the thicker threads were presumably derived by an elongation and partial despiralization of the heteropycnotic or chromocentric regions so clearly seen in intact resting nuclei. The thicker threads were more heavily charged with thymonucleic acid, as revealed by the Feulgen stain, than the finer strands. This does not necessarily mean that there is inherently a greater concentration of desoxyribose nucleic acid per unit length of these chromosomes. It is not at all unlikely that a more tightly coiled state exists in them, as shown by Coleman (10) in the case of the differentially condensed X-chromosomes of the *Acrididae* and by White (37) in the heteropycnotic X-chromosomes of the *Tettigonidae* and *Acrididae*.

On strictly morphological grounds it is difficult, if not impossible, to distinguish between chromosomes derived from normal, hyperplastic, or epidermal cells (Figs. 1 to 3). This is hardly remarkable since microscopic examination of the normal, hyperplastic and malignant cells shows the same types of nuclear architecture—chromatin strands and heteropycnotic masses on the so-called "net work" with the diameters of the intranuclear strands corresponding with that of the isolated fibrous chromatin. However, the chromosomes isolated from squamous cell carcinoma appear to be more densely stained with the Feulgen stain that those of the other tissues. This agrees with the cytochemical findings described below. Because of his failure to observe loose ends in intact resting nuclei, Claude and Potter (9) came to the conclusion that the chromatin threads were oriented end to end to form a continuous filament. We cannot agree; for chromosome threads are distributed in random fashion throughout the nuclear space and allowance has to be made for the fact that they represent structures spatially distributed in three dimensions. It would, therefore, be extremely difficult to detect free ends. Moreover, the weight of cytological evidence, gained by a study of the mitotic cycle, militates strongly against a continuous end to end alignment of chromosomes.

Cytochemistry.—The formed elements easily go into solution in 1 molar sodium chloride and in distilled water, leaving highly refractile granules. They may be reprecipitated in the form of fibrous material by the addition of water. Other physicochemical properties detected in fibrous nucleoproteins by Mirsky and Pollister (25, 26) and Mirsky (27) have also been observed but will not be described here.

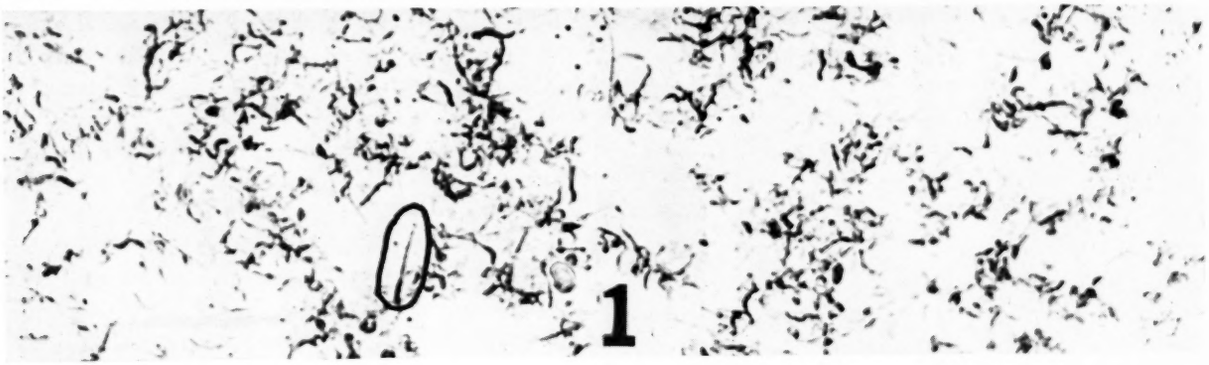
Significant differences were found in the desoxyribose nucleic acid content of the chromosomes of normal, hyperplastic and carcinomatous cells (Table I). It will be seen that the average content of desoxyribose nucleic acid in normal epidermal chromosomes is 0.129 mgm. per mgm. of dry weight or, roughly 13 per cent, whereas in mice painted thrice with methylcholanthrene it is 0.091 mgm. per mgm. of dry weight showing a 30 per cent drop in desoxyribose nucleic acid as compared with the normal. Increasing the number of paintings to 6 has no significant effect on the nucleic acid content of the chromosomes.

TABLE I: DESOXYRIBOSE NUCLEIC ACID OF CHROMOSOMES IN EXPERIMENTAL EPIDERMAL CARCINOGENESIS

No. of mice	No. of paintings	Time after first treatment to killing of mice: days	DNA* per mgm. dry wt.
		NORMAL	
13	—	—	0.108
11	—	—	0.136
12	—	—	0.154
10	—	—	0.118
46 (Total)		Average	0.129
		METHYLCHOLANTHRENE-TREATED MICE—HYPERPLASTIC	
8	3	10	0.098
10	3	10	0.083
11	3	11	0.090
12	3	11	0.092
41 (Total)		Average	0.091
11	6	16	0.091
17	6	16	0.099
12	7	16	0.127
40 (Total)		Average	0.106
		SQUAMOUS CELL CARCINOMA	
8	—	—	0.171
10	—	—	0.211
6	—	—	0.203
10	—	—	0.230
10	—	—	0.226
44 (Total)		Average	0.208

* DNA = Desoxyribose nucleic acid.

In malignancy, however, there is an increase which, although it is variable in the different animals examined, nevertheless reaches spectacular heights compared with chromosomes of the normal and hyperplastic epidermises. If, for instance, we exclude the average amount (0.171 mgm. per mgm. dry weight) of desoxyribose nucleic acid in squamous cell carcinoma obtained from the first 8 mice examined (Table I), the remaining instances



FIGS. 1-3

are found to maintain a level which is more than 60 per cent above that in the chromosomes of normal epidermis and certainly more than double the nucleic acid content of chromosomes for hyperplastic epidermis.

It must be pointed out, however, that in all such studies based on analyses of components of whole tissues, including averages of several samples, we are handicapped by not being able to recognize any differences that may be *localized* in the tissues. It is not inconceivable that a marked localized change in a few cells might be masked by including with them numerous others which are not altered in the same manner. Notwithstanding this, we believe that the general trend of changes in the desoxyribose nucleic acid content of normal, hyperplastic and carcinomatous epidermal tissues, as reflected in the newly established equilibrium, is sufficiently clear to warrant suggestive inferences.

DISCUSSION

The change that takes place in desoxyribose nucleic acid content of the chromosomes of hyperplastic epidermal cells and of squamous cell carcinoma from that of normal epidermis appears to be in conformity with the altered economy of the cell. It is not clear how methylcholanthrene operates to produce an altered equilibrium at the precancerous level. Nor is there as yet unequivocal evidence that breakdown products of methylcholanthrene, under investigation by W. L. Simpson in this hospital, are the causal agents in sequelae of chemical changes occurring in the cell. But there is a strong suggestion that in some manner the balance of euchromatin-heterochromatin is upset.

In a consideration of present status of the part played by nucleic acids in tumor production, the work of Caspersson and Santesson (7) stands out. These investigators obtained quantitative data, by using ultraviolet spectrophotometric and cyotchemical methods, on disturbances in endocellular chemical mechanisms in epithelial tumors and propound the thesis that the genetically inert heterochromatic segments of chromosomes have a significant role in carcinogenesis. They believe that, since heterochromatin is a special center for synthesis of cell protein, any quantitative disturbances in it would immediately affect the pace of cell growth and during mitoses cause cumulative disturbances in the division of euchromatic and heterochromatic segments. This in turn, is assumed to pave the way for further qualitative and quantitative changes leading in the direction of neoplastic growth.

In the light of the concepts formulated by Caspersson and Santesson, the early drop in desoxyribose nucleic acid in hyperplastic epidermal chromosomes may logically be explained in either of two ways. First, there may be a direct lowering of desoxyribose nucleic acid synthesis in the nuclei following methylcholanthrene treatment. The more probable alternative, however, is that the decreased desoxyribose nucleic acid is a consequence of its partial conversion into cytoplasmic ribose nucleic acid. Biesele's findings (3) on the pronounced increase of cytoplasmic ribose nucleic acid, even as early as half a day after methylcholanthrene treatment, bear this out. In the case of squamous cell carcinoma the desoxyribose nucleic acid content is conspicuously intensified. This again suggests several interpretations. First, there may be less conversion of the desoxyribose nucleic acid into the ribose form. Alternatively, it may mean a greater synthesis of the desoxyribose nucleic acid connected with more intense mitotic activity.

Whether the altered desoxyribose nucleic acid equilibrium in hyperplastic cells is the first step that sets in train a sequence of events in the cellular system on its way to malignancy, or whether it is merely an accompanying phenomenon along with the other occurrences, such as decreases in calcium, iron, copper, zinc and lipids, cholesterol, etc., is not evident. But in view of the importance of desoxyribose nucleic acid in chromosomal autonomy and heredity and since a cancer cell is, genetically speaking, distinct from a normal one, any event that modifies this hereditary constituent is likely to have far-reaching consequences. It is, therefore, not out of place to examine in this connection the work of other investigators who have analyzed this problem in the light of the euchromatin-heterochromatin balance.

The case of *Sorghum purpureo-sericeum* investigated by Darlington and Thomas (17) is particularly interesting on the theory that nucleic acid imbalance causes malignancy. In normal plants, according to these workers, the first division of the pollen grains gives a vegetative and a generative nucleus, the latter of which gives rise to the two male nuclei. But in plants where heterochromatic supernumerary B-chromosomes are

DESCRIPTION OF FIGURES 1 TO 3

The photomicrographs were made from Feulgen stained preparations and are magnified approximately 700 diameters. In the ringed areas, the morphology of chromosomes is distinctly seen. Many of the chromosomes show a differentiation into long and short arms, separated by Feulgen-negative gaps, which correspond to the position of the centromeres.

FIG. 1.—Chromosomes isolated from normal epidermis.

FIG. 2.—Isolated chromosomes from hyperplastic epidermis.

FIG. 3.—Isolated chromosome concentrate from squamous cell carcinoma.

present, the vegetative nucleus undergoes a succession of polymitotic divisions giving rise to several generative nuclei. To all intents and purposes the pollen grain behaves like an "encapsulated tumor." This exceptional behavior of the vegetative nucleus is presumably brought about by action of the supernumerary heterochromatic B-chromosomes. The activity of so-called inert chromosomes would, therefore, seem to indicate that nucleic acid supply is a crucial variable in the causation of neoplastic growth. From this viewpoint Darlington (18) visualizes in normal growth a correct euchromatin-heterochromatin balance. Morbid mitoses, he thinks, are produced when this balance is upset.

From a detailed cytological analysis of chromosome behavior in 565 human tumors (carcinoma of the skin, esophagus, colon, rectum, larynx, lung, cervix, uterus and breast) Koller (24) has come to similar conclusions. He maintains that the quantitative change in nucleic acid metabolism revealed by chromosome behavior may be one of the deciding criteria of distinction between normal cells and tumor cells derived from them. According to him the normal functioning of a cell is conditioned by a specific heterochromatin-euchromatin balance. Since the amount of nucleic acid and the rate of its production determine the frequency of division, Koller argues that there must be a *causal* connection between abnormally high concentrations of desoxyribose nucleic acid and intensified division rate and malignancy.

That there is an increase in the amount of Feulgen-positive material in rat sarcoma as compared with normal rat fibroblasts was histochemically shown by Cowdry (15) from a study of tissue culture of these cells. Stowell (35) devised a photometric histochemical method for the determination of thymonucleic acid in normal, hyperplastic and neoplastic epidermis of mice and men, and established the order of decreasing mean amount of thymonucleic acid per unit volume of tissue and per cell. Hyperplastic epidermis in which the cells were large, contained less thymonucleic acid per unit volume of tissue than the normal epidermis and the carcinomas contained more desoxyribose nucleic acid per unit volume of tissue than the normal epidermis. Using homologous normal and tumor tissues (rat liver, rat hepatoma, mouse lung and mouse lung tumors) Schneider (30) discovered that the desoxyribose nucleic acid content of both tumors was much higher than that of their normal homologues.

In contrast to the findings of the previous workers Dounce (23) found no increase in the desoxyribose nucleic acid of nuclei isolated from Walker carcinosarcoma 256, normal liver and rat hepatoma 31, but actually observed a decrease in the hepatoma cells. It is difficult to explain this apparent discrepancy unless we admit that it is due to differences in technic, and the types of tumors used. Moreover, as Stowell (35) has shown, concentrations of desoxyribose nucleic acid vary greatly in different types of tumor and even in different portions of the same tumor. In addition, the rates of division and of growth have a pronounced effect on the quantitative relationships of the nucleic acids in the tumors. Thus, a high rate of division with its accompanying disturbances of euchromatin-heterochromatin equilibrium is more likely to cause a succession of chromosomal aberrations than one where the rates of division and growth are slow (cf. Koller). It is now known from the work of Brachet (5), Caspersson and Schultz (8), Schultz (31), Darlington (18), Painter (28, 29) and others that, since desoxyribose nucleic acid is lowest in resting nuclei, the presumption is justified that its synthesis is also very low. It is maximal at metaphase and in tumors with a higher mitotic index there would be a corresponding increase of desoxyribose nucleic acid. It should also be emphasized that any disturbances in the mitotic mechanism are bound to have their impact on the dividing cells. This is in conformity with the sorghum-phenomenon of Darlington and Thomas referred to earlier.

Carruthers and Suntzeff (6) found in hyperplastic epidermis of mice treated with methylcholanthrene and in squamous cell carcinoma less desoxyribose nucleic acid than in normal or benzene-treated epidermis. This does not really conflict with the present findings since their analyses were based on unit weights of tissue and not on unit weights of chromosomes. It should also be remembered in this connection that the decrease in the desoxyribose nucleic acid is probably due to the increase in the size of the cells as demonstrated by Cowdry and Paletta (16).

On the basis of present experiments, we are unable either to support or refute the concept of a definite *causal connection* between disturbed nucleic acid balance and neoplastic growth. It is clear, however, that the decrease in the desoxyribose nucleic acid values taking place in hyperplastic epidermis is not necessarily dependent on the nucleocytoplasmic relationships of the cells. It is actual, as revealed by cytochemical determinations of chromosomes on a dry weight basis. In squamous cell carcinomas instead of a projection of the altered equilibrium so manifestly seen in hyperplastic epidermis, we witness a notable modification. The desoxyribose nucleic acid is sharply increased. In normality, the nuclear system appears to be in a state of dynamic equilibrium in which there is a regular and periodic removal and replacement of desoxy and ribose nucleic acids, respectively. In malignancy this equilibrium is violently upset and there is a greater accumulation of desoxyribose nucleic acid in the chromosomes, resulting in a multitudinous series of mitotic aberrations of the

type described by Koller (24) and Darlington and Thomas (17). From the cytochemical evidence at hand the conclusion that there is a constant association of increased desoxyribose nucleic acid with squamous cell carcinoma is thus inescapable and because nucleic acids are so intimately bound up with the genetic structure of the cellular system, it would, therefore, seem that quantitative changes in their attachment or detachment from the chromosomes assume more than trivial interest. It is irrelevant for our argument whether this augmentation in carcinoma is the result of greater storage or greater synthesis of desoxyribose nucleic acid or lesser conversion of the latter into the ribose type.

We have remarked earlier that there are not sufficiently discernible features between chromosomes isolated from normal and abnormal tissues to warrant a distinction on a purely morphological basis. This does not necessarily mean that there are no enlarged chromosomes in neoplastic tissues, as observed by Biesele and Cowdry (4). Since only a few cells are in mitosis in the hyperplastic and carcinomatous cells and fewer still in metaphase, it would not be a simple matter to detect enlarged chromosomes among the enormous number of threads presented by chromosome concentrates. However, the earlier observations of Biesele on chromosomal changes in epidermal carcinogenesis and the assumption of polytene (multiple strand) structure of neoplastic chromosomes need to be revised. A valid criterion of distinction between chromosomes isolated from different states of epidermis would be the recognition of quantitative differences in their desoxyribose nucleic acid content.

SUMMARY

1. A mechanical method of separation and isolation of chromosomes from the tissues of mouse epidermis in normality, hyperplasia and squamous cell carcinoma is described.

2. Cytochemical determinations of desoxyribose nucleic acid from the three stages in carcinogenesis are given. Soon after the application of methylcholanthrene there is seen a sharp drop in the concentration of desoxyribose nucleic acid in the chromosomes, which is maintained at a new equilibrium level until the onset of carcinoma. In this state there occurs a striking increase in the desoxyribose nucleic acid content of the chromosomes, far above that of chromosomes in normal and hyperplastic epidermises.

3. The bearing of the disturbances of nucleic acid equilibrium on carcinogenesis is discussed.

ACKNOWLEDGMENT

We wish to express our grateful thanks to Dr. C. Carruthers of this Hospital for his generous cooperation in the cytochemical aspect of this investigation. We are also appreciative of the many helpful suggestions offered by Dr. W. L. Simpson.

REFERENCES

1. Baumberger, J. P., Suntzeff, V., and Cowdry, E. V Methods for the Separation of Epidermis from Dermis and some Physiologic and Chemical Properties of Isolated Epidermis. J. Nat. Cancer Inst., **2**:413-423. 1942.
2. Bensley, R. R. Plasmosin. The Gel- and Fiber-Forming Constituent of the Protoplasm of the Hepatic Cell. Anat. Rec., **72**:351-369. 1938.
3. Biesele, J. J. Ribonucleic Acid and Heterchromatin in Epidermal Carcinogenesis. Cancer Research, **4**: 737-750. 1944.
4. Biesele, J. J., and Cowdry, E. V. Chromosomal Changes in Epidermal Carcinogenesis. J. Nat. Cancer Inst., **4**: 373-384. 1944.
5. Brachet, J. Recherches sur la synthèse de l'acide thymonucléique pendant le développement de l'oeuf d'Oursin. Arch. d. biol., **44**:519-576. 1933.
6. Carruthers, C., and Suntzeff, V. Desoxyribonucleic Acid in Epidermal Carcinogenesis Induced by Methylcholanthrene. Cancer Research, **6**:8-10. 1946.
7. Caspersson, T., and Santesson, L. Studies on Protein Metabolism in the Cells of Epithelial Tumors. Acta. Radiologica. Supp. **46**, 1942.
8. Caspersson, T., and Schultz, J. Nucleic Acid Metabolism of the Chromosomes in Relation to Gene Reproduction. Nature, London, **142**:294-295. 1938.
9. Claude, A., and Potter, J. S. Isolation of Chromatin Threads from the Resting Nucleus of Leukemic Cells. J. Exper. Med., **77**:345-354. 1943.
10. Coleman, L. C. Chromosome Structure in the Acrididae with Special Reference to the X-Chromosome. Genetics, **28**:2-8. 1943.
11. Cooper, Z. K., Firminger, H. I., and Reller, H. C. Transplantable Methylcholanthrene Skin Carcinomas of Mice. Cancer Research, **4**:617-621. 1944.
12. Cowdry, E. V. Experimental Epidermal Methylcholanthrene Carcinogenesis in Mice. Biol. Symposia, **10**:131-162. 1943.
13. Cowdry, E. V. Experimental Epidermal Methylcholanthrene Carcinogenesis in Mice. Second Summarizing Report. J. Invest. Dermat., **6**:15-42. 1945.
14. Cowdry, E. V. Microscopic and Chemical Properties of Precancerous Lesions. Science, **102**:165-168. 1945.
15. Cowdry, E. V. Results Secured by Applying the Feulgen Reaction to Fibroblasts and Sarcomatous Cells in Tissue Cultures. Science **68**:138-140. 1928.
16. Cowdry, E. V., and Paletta, F. X. Changes in Cellular, Nuclear and Nucleolar Sizes during Methylcholanthrene Epidermal Carcinogenesis. J. Nat. Cancer Inst., **1**:745-759. 1941.
17. Darlington, C. D., and Thomas, P. T. Morbid Mitosis and the Activity of Inert Chromosomes in Sorghum. Proc. Roy. Soc., s. B, **130**:127-150. 1941.
18. Darlington, C. D. Chromosome Chemistry and Gene Action. Nature, London, **149**:66-69. 1942.
19. Davidson, J. N. Nucleoproteins in Growth and Development. Edin. Med. J., **52**:344-356. 1945.
20. Davidson, J. N., and Waymouth, C. Nucleic Acids and Tissue Growth. Nutrition Abstracts and Review, **14**:1-18. 1944.

21. Dische, Z. Über einige neue charakteristische Farbreaktionen der Thymonukleinsäure und eine Mikromethode zur Bestimmung derselben in tierischen Organen mit Hilfe dieser Reaktionen. Mikrochemie, **8**:4-32. 1930.
22. Dounce, A. L. Further Studies on Isolated Cell Nuclei of Normal Rat Liver. J. Biol. Chem., **151**: 221-233. 1943.
23. Dounce, A. L. The Desoxyribonucleic Acid Content of Isolated Nuclei of Tumor Cells. J. Biol. Chem., **151**:235-240. 1943.
24. Koller, P. C. Origin of Malignant Tumor Cells. Nature, **151**:244-246. 1943.
25. Mirsky, A. E., and Pollister, A. W. Nucleoproteins of Cell Nuclei. Proc. Nat. Acad. Sc., **28**:344-352. 1942.
26. Mirsky, A. E., and Pollister, A. W. Fibrous Nucleoproteins of Chromatin. Biol. Symposia, **10**:247-260. 1943.
27. Mirsky, A. E. Chromosomes and Nucleoproteins. Advances Enzymol., **3**:1-34. 1943.
28. Painter, T. S., and Taylor, A. N. Nucleic Acid Storage in the Toad's Egg. Proc. Nat. Acad. Sc., **28**:311-317. 1942.
29. Painter, T. S. Cell Growth and Nucleic Acids in the Pollen of *Rhoeo discolor*. Bot. Gaz., **105**:58-68. 1943.
30. Schneider, W. C. Phosphorus Compounds in Animal Tissues. II. The Nucleic Acid Content of Homologous Normal and Cancer Tissues. Cancer Research, **5**:717-721. 1945.
31. Schultz, J. The Evidence of the Nucleoprotein Nature of the Gene. Cold Spring Harbor Symposia on Quant. Biol., **9**:55-65. 1941.
32. Seibert, Florence B. Removal of Impurities, Nucleic Acid and Polysaccharide from Tuberculin Protein. J. Biol. Chem., **133**:593-604. 1940.
33. Stowell, R. E. Thymonucleic Acid in Tumors. Cancer Research, **5**:283-294. 1945.
34. Stowell, R. E., and Cooper, Z. K. The Relative Thymonucleic Acid Content of Human Normal Epidermis, Hyperplastic Epidermis, and Epidermoid Carcinomas. Cancer Research, **5**:295-301. 1945.
35. Stowell, R. E. Photometric Histochemical Determination of Thymonucleic Acid in Experimental Epidermal Carcinogenesis. J. Nat. Cancer Inst., **3**:111-121. 1942.
36. Suntzeff, V., and Carruthers, C. The Water Content in the Epidermis of Mice undergoing Carcinogenesis by Methylcholanthrene. Cancer Research, **6**:574-577. 1946.
37. White, M. J. D. The Heteropycnosis of Sex Chromosomes and Its Interpretation in Terms of Spiral Structure. J. Genetics, **40**:67-82. 1940.
38. Ziegler, Dorothy M. A Method for the Collection of Nuclei of Epidermal Cells. Anat. Rec., **91**:169-173. 1945.

Succinic Dehydrogenase and Cytochrome Oxidase in Epidermal Carcinogenesis Induced by Methylcholanthrene in Mice*

Christopher Carruthers, Ph. D., and V. Suntzeff, M.D.

(From the Barnard Free Skin and Cancer Hospital, St. Louis 3, and the Department of Anatomy, Washington University School of Medicine, St. Louis 10, Missouri)

(Received for publication June 28, 1946)

The role of the alkalies, alkaline earths, and of iron, copper, and zinc in epidermal carcinogenesis in mice has been described (4) and the integration of the chemical, physical, and histological changes in mouse epidermis undergoing carcinogenesis has been reviewed by Cowdry (7). The investigations on the minerals revealed that the calcium, iron, zinc, and copper contents of hyperplastic (methylcholanthrene-treated) epidermis were significantly less than that of normal epidermis, and furthermore the content of these metals in the squamous cell carcinoma was reduced to even lower values. Since calcium may be associated with the succinic dehydrogenase system (15, 21, 27), and copper and iron with cytochrome oxidase (20), experiments were undertaken to study these enzymes in the epidermis of mice under the carcinogenic influence of methylcholanthrene.

EXPERIMENTAL

Swiss mice of both sexes were painted with methylcholanthrene (0.6 per cent weight per volume in reagent grade benzene) as in our previous work (4). The mice were sacrificed 5 days after the last application of either benzene or carcinogen. The epidermis of normal controls, benzene-treated controls, and of methylcholanthrene-treated mice was removed from the dermis at 41° to 42° C. on a constant temperature hot plate, or it was scraped off at room temperature.[1] For the final stage in our carcinogenic series, we used the transplantable squamous cell carcinoma of Cooper, Firminger, and Reller (6). This tumor has now passed through 40 generations and has remained practically the same morphologically.

After removal of the various specimens of treated epidermises, they were immediately dropped into a test tube (submerged in an ice bath) containing 1 cc. of redistilled water, and after weighing, the tissue was homogenized by the technic of Potter and Elvehjem (16). The carcinomas were first ground in an unglazed mortar and then homogenized. Succinic dehydrogenase and cytochrome oxidase were determined by the method of Schneider and Potter (21) upon the tissue homogenate at a concentration of 10 per cent. As recommended by Potter, succinic dehydrogenase was tested at two different tissue concentrations, and cytochrome oxidase at three, from which the auto-oxidation of ascorbic acid was determined.[2]

In our previous investigations on the role of the minerals in epidermal carcinogenesis, the basis of reference was nucleoprotein phosphorus (4). Since the nucleoprotein phosphorus content is nearly the same in normal and hyperplastic epidermis and in the transplantable squamous cell carcinoma used in our investigations (23), the conventional method used by practically all workers in enzymology of expressing the results on a dry weight basis was employed. Moreover, the extent of the decrease in the calcium, copper, and zinc contents in hyperplastic epidermis is the same whether nucleoprotein phosphorus or the wet weight of the tissue is selected as a basis of reference (23-25). Also, the decrease in the total lipid is practically the same when lipid protein or the dry weight of the fat-free tissue is employed as a basis of reference (26). It would, therefore, appear that the diminution of some of the chemical constituents in hyperplastic mouse epidermis was independent of the basis of reference. Although the same premise might not hold for the activity of the enzymes, the dry weight appeared to be as good a basis of reference as any. The increase in the activity of succinic dehydrogenase in the carcinoma and of cytochrome oxidase in late hyperplastic epidermis and in the carcinoma may be partially explained on a morphological basis since

* Aided by grants from the National Cancer Institute, the International Cancer Research Foundation and an anonymous donor.

[1] Unpublished experiments.

[2] The authors are indebted to Dr. Van R. Potter of McArdle Memorial Laboratory, Madison 6, Wisconsin, for the assay of two samples of cytochrome c.

Cowdry and Paletta (8) have shown that the cell volume of both spinous and basal cells shows a progressive increase as the hyperplasia progresses, and decreases in the carcinoma. The latter were induced by methylcholanthrene and are not strictly comparable to the transplantable tumor employed by us. The same authors have also demonstrated that the nucleocytoplasmic ratio of both spinous and basal cells decreases in hyperplastic epidermis and increases in the carcinoma, both being less than that of normal epidermis.

RESULTS

The results on normal untreated, benzene-treated, and methylcholanthrene-treated epidermises are shown in Table I and are expressed in the usual way, that is, micro-liters of oxygen consumed per mgm. dry weight per hour. The Q_{O_2} values for cytochrome oxidase activity were corrected for the degree of homogenization of the tissue according to the method of Schneider and Potter (21). In all of our previous investigations the epidermis was removed at 50° to 51° C. (2), but this temperature was found to have a destructive effect upon succinic dehydrogenase and cytochrome oxidase activities in the epidermis of young mice whose dermis is quite thin and permits too much heat to pass to the epidermis. However the epidermis removed at 41° to 42° C. gave $Q^{s}_{O_2}$ (succinic dehydrogenase activity) and $Q^{ox}_{O_2}$ (cytochrome oxidase activity) values of 3.9 and 25.0 respectively which agreed very well with $Q^{s}_{O_2}$ of 3.6 and $Q^{ox}_{O_2}$ of 24.8 for epidermis removed at room temperature. The epidermis was either scraped off at room temperature[1] or removed at 41° to 42° C. for the determinations reported here. Two groups of mice about 1 year of age gave $Q^{s}_{O_2}$ of 2.8 and $Q^{ox}_{O_2}$ of 22.2 which values are somewhat lower than those for the normal young mice.

TABLE I: SUCCINIC DEHYDROGENASE AND CYTOCHROME OXIDASE IN EPIDERMAL CARCINOGENESIS

Q_{O_2} (cu. mm. O_2 per mgm. dry weight per hour)

Treatment	No. of mice	Succinic dehydrogenase	Cytochrome oxidase
Epidermis removed at room temperature by			
		$Q^{s}_{O_2}$	$Q^{ox}_{O_2}$
scraping	10	3.1	24.7
"	10	4.8	23.8
"	10	3.0	25.8
	30	Average 3.6	24.8
Epidermis removed at 41-42°	9	4.4	25.2
"	12	3.0	25.8
"	14	4.9	24.9
"	10	2.8	29.5
"	10	4.6	23.5
"	12	3.8	21.1
	67	Average 3.9	25.0
Epidermis of 1 year old mice removed at 41-42°	10	3.0	21.8
"	10	2.6	22.6
	20	Average 2.8	22.2
3 Paintings of Benzene	12	2.3	26.0
"	13	2.8	19.6
"	14	2.2	19.3
"	12	3.2	24.8
"	14	2.8	19.8
	65	Average 2.7	21.9
6 Paintings of Benzene	11	3.2	26.9
"	15	2.8	22.7
"	14	2.2	28.1
"	13	2.0	17.6
	53	Average 2.5	23.8
3 Paintings Methylcholanthrene	8	3.2	20.1[3]
"	9	4.3	23.1[4]
"	8	3.5	28.8
"	8	4.1	30.9
	33	Average 3.8	25.7
6 Paintings Methylcholanthrene.	10	3.6	33.6
"	8	4.1	29.0
"	8	5.1	31.7
"	10	3.0	28.5
	36	Average 3.9	30.7
12 Paintings Methylcholanthrene	16	3.4	30.5
"	10	3.5	33.7
"	12	4.0	30.7
"	11	3.7	27.4
	49	Average 3.6	30.6
18 Paintings Methylcholanthrene	7	3.5	45.6
"	8	3.6	43.3
"	10	3.5	45.8
	25	Average 3.5	44.9
24 Paintings Methylcholanthrene	13	3.7	54.3
"	8	3.4	36.2
"	6	3.9	52.9
"	10	4.2	47.5
"	7	4.0	49.9
	44	Average 3.6	48.2

[3], [4] Epidermis removed at 3 and 4 days respectively after the last application of methylcholanthrene.

Since benzene is used as a solvent for the carcinogen, its effect upon the enzymes was studied. Mice which received 3 applications of benzene on alternate days were sacrificed 5 days after the last treatment (all benzene and methylcholanthrene-treated mice were killed 5 days after the last application of either benzene or of the carcinogen) had a $Q^{s}_{O_2}$ value of 2.7 and $Q^{ox}_{O_2}$ value of 21.9 while the epidermis treated with 6 paintings of benzene on alternate days showed a $Q^{s}_{O_2}$ value of 2.5 and a $Q^{ox}_{O_2}$ value of 23.8. Therefore benzene alone has a slight inhibitory effect upon the activity of

these enzymes. Benzene was not applied over a longer period of time since methylcholanthrene was found to overcome the inhibitory effect of the solvent.

Mice which had received 3 applications of the carcinogen (applied on alternate days to all the following groups of mice) showed a $Q^{s}_{O_2}$ value of 3.8 and a $Q^{ox}_{O_2}$ value of 25.7 which are the same as those of normal epidermis. Six and 12 treatments of the epidermis did not appreciably alter the $Q^{s}_{O_2}$ values, but raised the $Q^{ox}_{O_2}$ values to 30.7 and 30.6 respectively. Epidermises treated over a longer period of time with 18 and 24 paintings of the carcinogen had $Q^{s}_{O_2}$ values of 3.5 and 3.6 respectively which are essentially the same as normal, but the $Q^{ox}_{O_2}$ values had risen to 44.9 and 48.2 respectively. The late hyperplastic epidermises, therefore, contained nearly twice as much cytochrome oxidase activity as did the normal.

The succinic dehydrogenase and cytochrome oxidase activities of the transplantable squamous cell carcinoma are shown in Table II. The results are expressed in the usual way under "dry weight" and also under "total solids." Since we have recently been able to remove the epidermis at room temperature,[1] it has been possible to determine the water content of normal, and hyperplastic epidermis and to compare the water content of the latter with the transplantable carcinoma. These studies showed that the water content of the carcinoma was 22 per cent greater than that of normal.[1] Under the caption "total solids" the dry weight of the tumors in each sample has been increased by 22 per cent to compensate for the increased amount of water. The average $Q^{s}_{O_2}$ value for the tumors was 16.1 and under "total solids" 13.1, which values are respectively about 4.5 and 3.5 times greater than that of normal. The average $Q^{s}_{O_2}$ value for the tumors was 41.6 and under "total solids" 13.1, which values are respectively about late hyperplastic epidermis, but greater than that of normal.

TABLE II: SUCCINIC DEHYDROGENASE AND CYTOCHROME OXIDASE IN EPIDERMAL CARCINOGENESIS

Q_{O_2} (cu. mm. O_2 per mgm. dry weight per hour)

	Succinic dehydrogenase		Cytochrome oxidase	
Tissue	Dry weight	Total solids	Dry weight	Total solids
Tumors	Q_{O_2}	Q_{O_2}	Q_{O_2}	Q_{O_2}
"	12.3	10.0	43.9	36.1
"	17.6	14.2	43.4	35.5
"	13.2	10.8	45.0	37.1
"	11.7	9.2	38.4	31.5
"	15.7	12.1	36.7	30.0
"	18.7	15.3	43.3	35.6
"	21.3	17.9	42.5	34.4
"	18.3	15.0	40.2	32.8
Average	16.1	13.1	41.6	34.1

DISCUSSION

The results on succinic dehydrogenase and cytochrome oxidase activity and of the metals, calcium, copper, iron, and zinc are shown graphically in Fig. 1 where the percentage of change in the metal nucleoprotein phosphorus ratios is plotted against the time and the number of applications of the carcinogen and where the Q_{O_2} is likewise plotted against the latter two variables. It can be seen that there is no change in the activity of succinic dehydrogenase until the epidermal cells become carcinomatous when it is increased signally. The investigations of Swingle, Axelrod, and Elvehjem (27) showed that calcium stimulates the succinic dehydrogenase system in homogenates. Potter and Schneider (15) have furthermore demonstrated that the activating effect of added calcium and aluminum to the homogenates is probably due to a multiple dilution effect, caused by the dissociation of cytochrome c, aluminum ions, and calcium ions away from the succinic dehydrogenase and cytochrome oxidase. Later Schneider and Potter (21) postulated that the necessity of calcium and aluminum for maximum activity of the succinic dehydrogenase system, and of aluminum for the cytochrome oxidase system was probably indirect.

In this study (Fig. 1) a decrease of nearly 60 per cent in the calcium nucleoprotein phosphorus ratio in the hyperplastic epidermis had no effect upon the activity of succinic dehydrogenase. However, in the tumors which are very low in calcium, the succinic dehydrogenase activity is high. It would, therefore, appear that either the effect of the tissue calcium was indirect or that sufficient calcium was added to the medium to compensate for the loss induced by the carcinogen. Since Potter and DuBois have shown that zinc has an inhibitory effect upon succinic dehydrogenase activity (17), perhaps the decrease in the zinc content of hyperplastic epidermis and in the tumors may account for an increased activity of this enzyme, or zinc may play an important role in its regulation.

An inspection of Fig. 1 reveals that the epidermis which had received 6 and 12 applications of the carcinogen has a greater cytochrome oxidase activity than normal. Moreover, this increase is pronounced in the late hyperplasia (18 and 24 paintings) at which time the papillomas and carcinomas begin to appear. (Our samples of late hyperplastic epidermis did not contain any papillomas or carcinomas.) In the carcinoma the activity of cytochrome oxidase is less than in late hyperplasia, but it is greater than that of the normal. Since cytochrome oxidase contains iron and since copper is essential for the formation and maintenance of cytochrome oxidase activity of heart muscle in rats and probably other tissues (20), an explanation for the increase in the activity of this enzyme where the copper and iron contents are decreased is not easy. However, the regulation of the cytochrome oxidase activity may be conditioned by the quantity of iron and copper in the

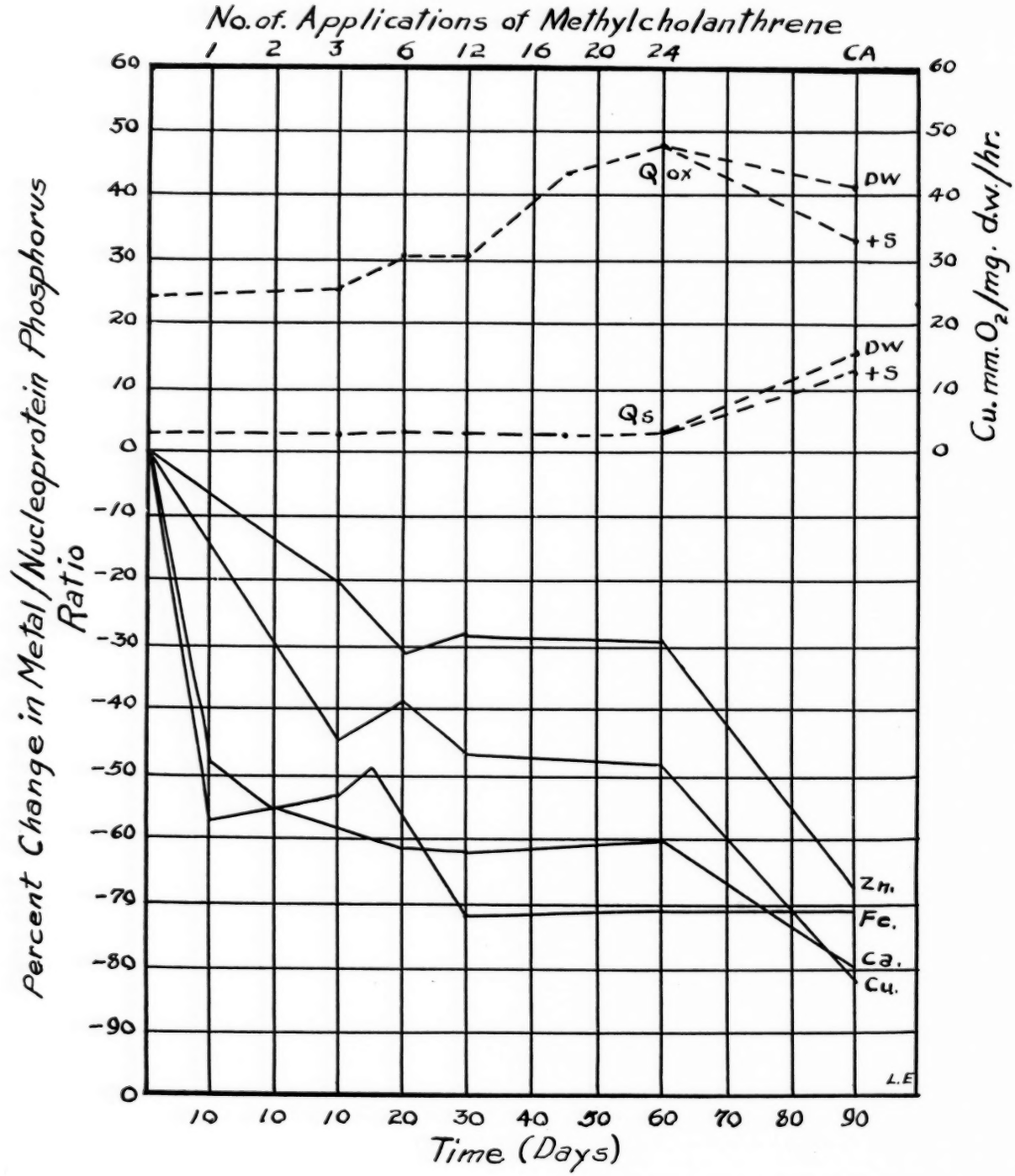


FIG. 1.—Succinic dehydrogenase, cytochrome oxidase, iron, calcium, copper, and zinc in epidermal carcinogenesis induced by methylcholanthrene. CA represents carcinoma.

cells. Fig. 1 also reveals that up to 30 days the contents of the metals are decreasing with time, and are in a state of some flux, and during this interval there is no appreciable change in the enzymes. However, from 30 to 60 days there is no further decrease in the metal contents, and it is during this time that the cytochrome oxidase activity begins to rise which would indicate a definite change in the cells prior to malignancy, in which state the activity of this enzyme decreases somewhat.

Care was taken to avoid necrotic tissue in the transplantable carcinoma since Elliott and Greig (11), the first to show that tumors were low in succinic dehydrogenase, later demonstrated that necrotic tumor tissue had an inhibitory effect upon this enzyme (12). How-

ever, Albaum and Potter showed that fresh tumor tissue free of necrotic material was amply adequate for the assay of this enzyme, but that autolysis produced inhibitory substances (1).

It is impossible here to relate our studies with the investigations of others on many types of tumors, so only those investigations pertaining to skin will be considered. In general, the activity of succinic dehydrogenase and cytochrome oxidase is less in tumors than in their normal prototypes (13, 22). Dickens and Weil-Malherbe (10) found that the respiration and glycolysis of carcinoma of the vulva were typical of those of malignant growths which have a high aerobic and anaerobic glycolysis. Leukoplakia showed only a slight increase in the latter when compared with normal skin. However, these investigators used whole skin for the normal, a large part of which is dermis, so that the analyses do not relate, as ours do, specifically to epidermis.

Salter and his co-workers (9) have found that the Q_{O_2} of tumor tissues in general increases less in response to succinate and *p*-phenylenediamine than does the Q_{O_2} of normal tissue. These investigators found that virus-induced papillomas removed at 6 and 7 weeks gave a 2 to 3 fold increase in Q_{O_2} response to succinate and *p*-phenylenediamine whereas the virus-induced papillomas removed at 10 and 14 weeks and described as benign did not show an increase. This observation indicated to these workers that the papilloma had begun to behave like a malignant growth. In a subsequent paper, Salter and his colleagues removed tissues at 46, 55, and 79 weeks, the first specimens being benign papillomas, and the latter carcinomatous (19). All showed a loss of cytochrome system response to the substrates succinate and *p*-phenylenediamine. Kidd, Winzler, and Burk have made similar observations on virus-induced tumors of the rabbit, and have found that normal rabbit skin gave a greater Q_{O_2} response to succinate and *p*-phenylenediamine than did V-2 carcinoma (14). The virus-induced Shope papilloma gave the same low Q_{O_2} response to the substrates as did the V-2 carcinoma. These investigators also demonstrated that the metabolism of the V-2 carcinoma is characteristic of malignant cells generally.

On the other hand, Berenblum, Chain, and Heatley (3), using rabbit epidermis, observed no differences in oxygen uptake, aerobic and anaerobic glycolysis, and RQ of normal epidermis and a Shope virus papilloma. The values they obtained for normal epidermis were nearly identical with those of the papilloma, and very similar to the values cited in the literature for squamous carcinoma.

Rosenthal and Drabkin, in a study of the response of normal and malignant tissues to succinate and *p*-phenylenediamine, reported (18) that normal epithelial tissues fall into two groups as follows: (a) tissues with a high oxidative response towards the substrates (kidney cortex, liver, brain cortex, and probably smooth and striated muscles); (b) tissues with low response towards the substrates (gastrointestinal mucosa, lung, and possibly skin, mammary gland, and lymphatic tissue). Benign and malignant rat tumors and human carcinomas exhibited low oxidative responses similar to group (b). These investigators claim that the oxidative behavior incident to malignancy can be expected to occur only in group (a), where spontaneous tumors rarely occur.

In contrast to the observations cited above, we have found that the activity of cytochrome oxidase in late hyperplastic mouse epidermis is greater than normal, but less than the squamous cell carcinoma, and that the activity of succinic dehydrogenase is unchanged from the normal in hyperplastic epidermis, but increases significantly in the carcinoma.

In a comparison of the chemical changes in the early stages of carcinogenesis with tissue found in a carcinomatous tumor, it must be remembered that the cellular material analyzed in a carcinoma, which consists mainly of cancer cells together with some stroma, is more homogeneous than the cellular material presented by the several samples of epidermis obtained from the skin of 8 to 12 different mice during the intermediate stages of carcinogenesis. In this latter material, the skins of individual mice show considerable variation in the degree of chemical hyperplasia. Moreover, since cancer arises always in a sharply circumscribed area of a treated epidermis, there are considerable differences of the degree of chemically precancerous hyperplasia in the same skin, and the increase in cytochrome oxidase in this late hyperplasia may be due to the increase in the number of scattered foci where cancer is about to originate.

SUMMARY

Succinic dehydrogenase and cytochrome oxidase activity of mouse epidermis undergoing carcinogenesis by methylcholanthrene has been investigated. The activity of succinic dehydrogenase in the normal and hyperplastic epidermis is the same, but almost a four-fold increase occurs when the cells become carcinomatous. Cytochrome oxidase activity in early hyperplastic epidermis (6 and 12 paintings of methylcholanthrene) was slightly greater than normal, but the activity of late hyperplastic epidermis (18 and 24 applications of the carcinogen) was nearly twice that of normal. In the carcinoma the activity of this enzyme was less than that of late hyperplastic epidermis, but greater than that of the normal. The relationship of the enzymes to the metals, calcium, iron, copper, and zinc, is briefly discussed.

REFERENCES

1. Albaum, H. G., and Potter, V. R. Biocatalysts in Cancer Tissue. II. Inhibition of the Succinoxidase System by Tumor Extracts. Cancer Research, **3**:303-308. 1943.
2. Baumberger, J. P., Suntzeff, V., and Cowdry, E. V. Methods for the Separation of Epidermis from Dermis and Some Physiologic and Chemical Properties of Isolated Epidermis. J. Nat. Cancer Inst., **2**:413-423. 1942.
3. Perenblum, I., Chain, E., and Heatley, N. G. The Metabolism of Normal and Neoplastic Skin Epithelium. Evidence against the Theory that Aerobic Glycolysis Associated with a Low R. Q. Is a Feature of a Disturbed Metabolism Indicative of Tumour Growth. Am. J. Cancer, **38**:367-371. 1940.
4. Carruthers, C., and Suntzeff, V. Copper and Zinc in Epidermal Carcinogenesis Induced by Methylcholanthrene. J. Biol. Chem., **159**:647-651. 1945.
5. Carruthers, C., Cowdry, E. V., and Suntzeff, V. Influence of Age on Copper and Zinc in Epidermal Carcinogenesis Induced by Methylcholanthrene in Mice. Manuscript in preparation.
6. Cooper, Z. K., Firminger, H. I., and Reller, H. C. Transplantable Methylcholanthrene Skin Carcinomas of Mice. Cancer Research, **4**:617-621. 1944.
7. Cowdry, E. V. Experimental Epidermal Methylcholanthrene Carcinogenesis in Mice. Second Summarizing Report. J. Invest. Dermat., **6**:15-42. 1945.
8. Cowdry, E. V., and Paletta, F. X. Changes in Cellular, Nuclear, and Nucleolar Sizes during Methylcholanthrene Epidermal Carcinogenesis. J. Nat. Cancer Inst., **1**:745-759. 1941.
9. Craig, F. N., Bassett, A. M., and Salter, W. T. Artificial Benignancy of Neoplasm. VI. Observations on the Oxidative Behavior of Tumors, Artificially Benign Tumors, and Homologous Normal Tissues. Cancer Research, **1**:869-879. 1941.
10. Dickens, F., and Weil-Malherbe, H. The Metabolism of Normal and Tumor Tissue. XX. A Comparison of the Metabolism of Tumors of Liver and Skin with that of the Tissue of Origin. Cancer Research, **3**:73-87. 1943.
11. Elliott, K. A. C., and Greig, M. E. The Distribution of the Succinic Oxidase System in Animal Tissues. Biochem. J., **32**:1407-1423. 1938.
12. Elliott, K. A. C. Inhibition of the Succinoxidase System by Extracts of Tumour and Normal Tissues. Biochem. J., **34**:1134-1141. 1940.
13. Greenstein, J. P. A.A.A.S. Research Conference on Cancer. 1945, p. 192.
14. Kidd, J. G., Winzler, R. J., and Burk, D. Comparative Glycolytic and Respiratory Metabolism of Homologous Normal, Benign, and Malignant Rabbit Tissues. Cancer Research, **4**:547-553. 1944.
15. Potter, V. R., and Schneider, W. C. Studies on the Mechanism of Hydrogen Transport in Animal Tissues. V. Dilution Effects in the Succinoxidase System. J. Biol. Chem., **142**:543-555. 1942.
16. Potter, V. R., and Elvehjem, C. A. A Modified Method for the Study of Tissue Oxidations. J. Biol. Chem., **114**:495-504. 1936.
17. Potter, V. R., and DuBois, K. P. Studies on the Mechanism of Hydrogen Transport in Animal Tissues. VI. Inhibitor Studies of Succinic Dehydrogenase. J. Gen. Physiol., **26**:391-404. 1943.
18. Rosenthal, O., and Drabkin, D. L. The Oxidative Response of Normal and Neoplastic Tissues to Succinate and to *p*-Phenylenediamine. Cancer Research, **4**:487-494. 1944.
19. Roskelley, R. G., Mayer, N., Horwitt, B. N., and Salter, W. T. Studies in Cancer. VII. Enzyme Deficiency in Human and Experimental Cancer. J. Clin. Investigation, **22**:743-751. 1943.
20. Schultze, M. O. The Effect of Deficiences in Copper and Iron on the Cytochrome Oxidase of Rat Tissues. J. Biol. Chem., **129**:729-737. 1939.
21. Schneider, W. C., and Potter, V. R. The Assay of Animal Tissues for Respiratory Enzymes. II. Succinic Dehydrogenase and Cytochrome Oxidase. J. Biol. Chem., **149**:217-227. 1943.
22. Schneider, W. C., and Potter, V. R. Biocatalysts in Cancer Tissue. III. Succinic Dehydrogenase and Cytochrome Oxidase. Cancer Research, **3**:353-357. 1943.
23. Suntzeff, V., and Carruthers, C. Potassium and Calcium in Epidermal Carcinogenesis Induced by Methylcholanthrene. J. Biol. Chem., **153**:521-527. 1944.
24. Suntzeff, V., and Carruthers, C. The Effect of Methylcholanthrene upon Epidermal Sodium and Calcium. Cancer Research, **3**:431-433. 1943.
25. Suntzeff, V., Carruthers, C., and Cowdry, E. V. Influence of Age on Calcium in Epidermal Carcinogenesis Induced by Methylcholanthrene in Mice. Cancer Research, **5**:572-575. 1945.
26. Suntzeff, V., Cowdry, E. V., and Carruthers, C. Influence of Age on Total Epidermal Lipid During Carcinogenesis Induced by Methylcholanthrene in Mice. Cancer Research, **6**:179-182. 1946.
27. Swingle, K. F., Axelrod, A. E., and Elvehjem, C. A. The Mechanism of the Effect of Calcium Salts on the Succinoxidase System. J. Biol. Chem., **145**:581-591. 1942.

Growth of Avian Tumors Other Than the Rous Sarcoma in the Anterior Chamber of the Guinea Pig Eye*

Edward W. Shrigley, Ph.D., M.D., Harry S. N. Greene, M.D., and F. Duran-Reynals, M.D.

(From the Departments of Bacteriology and Immunology, Pathology, and Surgery, Yale University School of Medicine, New Haven 11, Connecticut)

(Received for publication August 9, 1946)

The transplantation of the Rous chicken sarcoma to the eyes of guinea pigs and the variations in the behavior of the inciting agent following residence in this host have been described (9). During the course of these studies the heterotransplantability of several other avian tumors, both spontaneous and induced, was tested and the present paper deals with the results obtained.

MATERIALS AND METHODS

Two spontaneous and 3 chemically-induced tumors were selected for study. The spontaneous growths originated in chickens and have been referred to as "chicken tumor C" and "chicken tumor E" (3). One of the experimental tumors resulted from the injection of methylcholanthrene into chickens while the other growths were derived from guinea fowl and pigeons, respectively, in a similar manner (4). Guinea pigs were used as the alien host and the anterior chamber of the eye as the inoculation site. The technic of transplantation employed has been adequately described (9). The tissue fragments were examined at frequent intervals and histological sections were obtained after death of the rodent. Growth was determined by increase in size of the transplanted tissue and histological similarity with the original tumor.[1] In two cases the occurrence of takes was further evidenced by the growth of fragments subsequently transferred from pigs to the species of origin. Unfortunately the present data are not extensive enough to determine the effect of the foreign host on the specificities of the tumor virus when such agents were present.

RESULTS

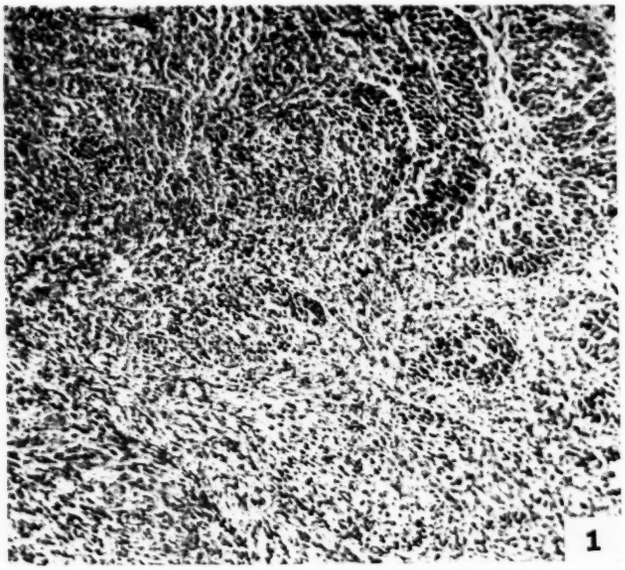
Methylcholanthrene-induced guinea fowl tumor.— The occurrence and characteristics of fibrosarcomas induced in guinea fowl by the injection of methylcholanthrene have been discussed (4). One such tumor has been successfully transplanted to chickens and carried serially in this species. After the fifth passage in these birds, Berkefeld "N" filtrates were obtained that produced tumors in chickens but not in guinea fowl (2). A question arises, therefore, with reference to the origin of this growth, *i.e.* is this a chemically induced guinea fowl tumor which has become filterable and lost its affinity for the species of origin, or is it actually a chicken tumor of virus etiology arising spontaneously? Further study is necessary before these questions can be answered.

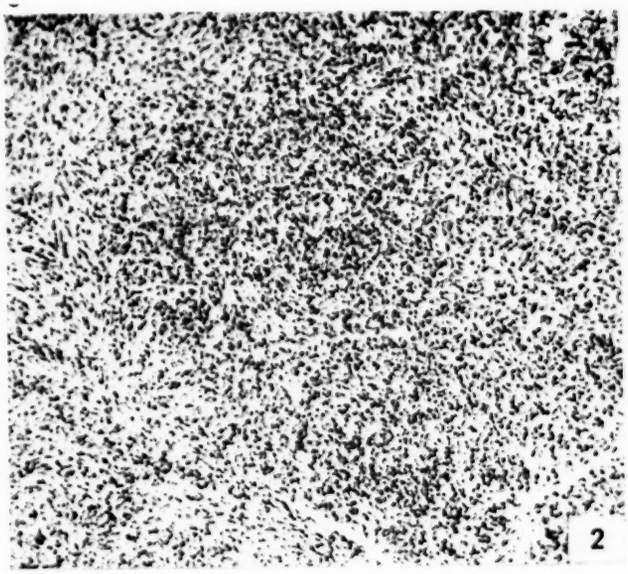
In the chicken this tumor is of moderate firmness, viscid and at times hemorrhagic, particularly in the older lesions. Filtrates injected into young chicks produce a hemorrhagic disease as has been described in relation to the Rous sarcoma virus (1, 7). Fig. 1 shows the cellular structure of this tumor. It can be seen that elongated fibroblasts resulting in a "streaming" type of architecture predominate. In the looser portions of the growth the cells tend to be oval and the amount of intercellular substance is increased. Mitotic figures are moderately abundant and there are areas in which the tumor cells invade muscle bundles.

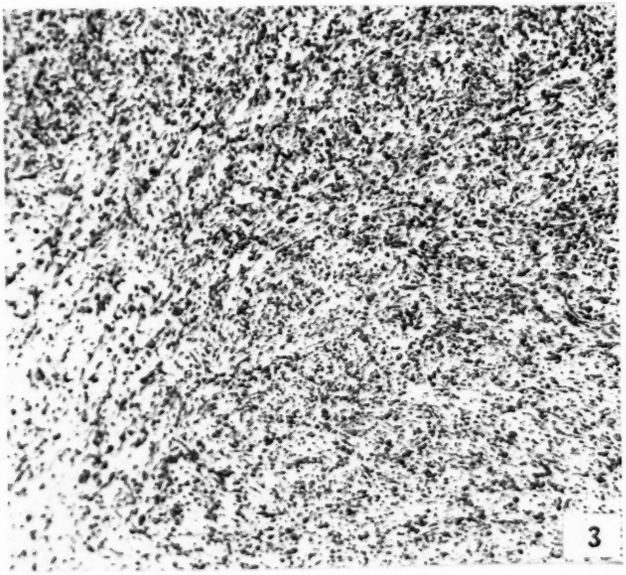
Transfer of the tumor to the guinea pig eye results in takes, but regression invariably occurs before the transplant fills the chamber. As a rule regression becomes evident from about the tenth to the 12th day after transfer and proceeds until the fragment disappears. The appearance of a guinea pig transplant on the 14th day is shown in Fig. 2. Here the tissue is dense with a central area of necrosis. There are mitoses in fair number, the cells being less elongated than in the original sarcoma. After reinoculation of the transplants into chicks one finds histologically a tumor that is quite similar to the one seen in the original chicken passage (Fig. 3). Injection of guinea fowl was not attempted. The growths following the inoculation of the guinea-pig-passage material into chicks developed in a week to 10 days, and in one instance hemorrhagic disease appeared. This suggests that the etiologic agent

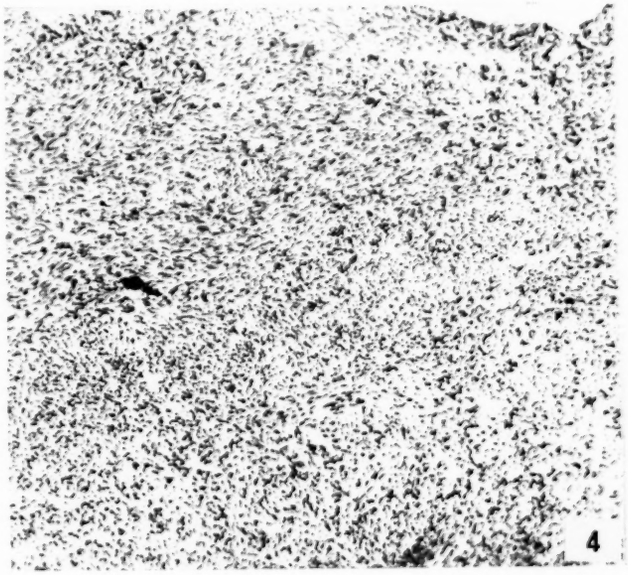
* This investigation was aided by grants from The Donner Foundation, Inc., and from The Jane Coffin Childs Memorial Fund for Medical Research.

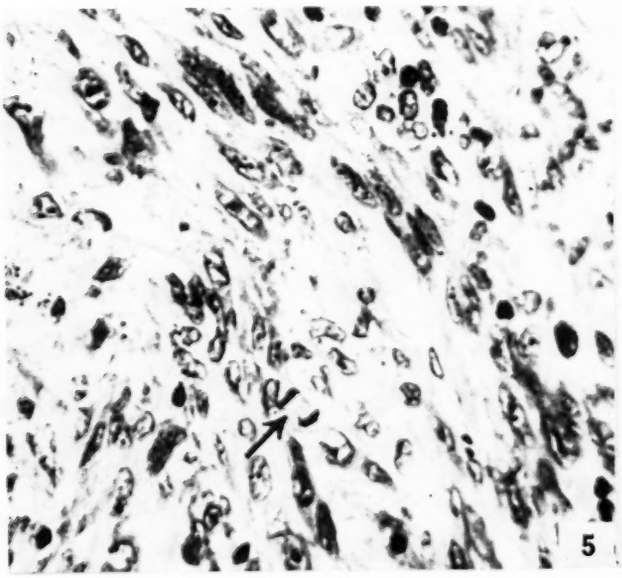
[1] In the transfer of extremely anaplastic tumors into heterologous hosts, differentiation sometimes occurs so that the histology of the growth in the guinea pig eye differs from that seen in the original tissue (6).

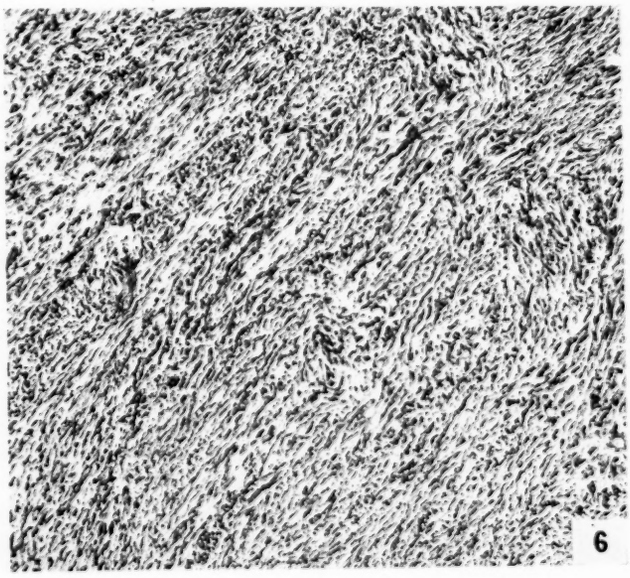












FIGS. 1-6

of this tumor had survived the sojourn in the guinea pig. Subsequent passages in birds did not reveal any alteration in the specificities of this agent. However, only 3 transfers in chickens were made.

Methylcholanthrene-induced pigeon tumor.—Sarcomas have been produced in pigeons by the injection of methylcholanthrene dissolved in benzene. One of these growths has been transferred in pigeons through 9 passages by cell suspensions; filtrates have been found inactive (4). The histological picture of this tumor from the pigeons is shown in Fig. 4. It will be seen that the architecture is much like the previous growth. The fibroblasts tend to be a little more elongated and there are small areas of necrosis present. Oval cells replace the thin fibroblasts in the looser areas of the sarcoma. Mitoses are occasional. Fig. 5 shows a high magnification of the same tumor after 19 days' residence in the anterior chamber of the guinea pig eye. Here there is some evidence of hyalin change in the center of the transplant with thin fibroblasts on the periphery. The tumor is well vascularized and cells in mitosis are numerous. Pigeons were not inoculated with this tissue from the rodent.

Methylcholanthrene-induced chicken tumor.—The tumor used for this transfer was obtained from an experiment designed to study the effect of vaccine virus infection upon methylcholanthrene tumors. Chicks were inoculated into the back of the neck with 10 mgm. of the carcinogen in sesame oil and later scarified on the breast with active vaccinia virus. One line of growths was started from a sarcoma which developed on the neck of a 10 months old bird so treated, and has been carried by cell suspensions for 77 passages in chicks. Active filtrates have never been obtained from these growths.

Fig. 6 shows the general histology of this tumor line after 26 passages. The picture is similar to the other two growths described above having relatively compact bands of elongated fibroblasts and less dense portions of oval-shaped cells. Certain areas show a considerable quantity of intercellular material, and mitoses are numerous. This tumor likewise invades muscle bundles. After 9 days in the guinea pig eye, there is a greater abundance of the oval cells and a considerable quantity of collagen. Only a few mitotic figures are present (Fig. 7). No chickens were inoculated with the rodent-passage material.

Spontaneous chicken tumor "C".—This sarcoma arose spontaneously in a Rhode Island Red hen and has been characterized through 21 chicken passages by its tough fibrous consistency and slow growth rate. Numerous active filtrates of this tumor have been obtained, and its general properties have been adequately described (3).

The histology of this sarcoma is so typical that it can easily be differentiated from other growths. Fig. 8 shows the very dense bands of attenuated fibroblasts of which this neoplasm is composed. Even in the more loose portions of the growth the cells are elongated. Muscle bundles are invaded, but mitoses are only occasionally seen. The appearance of the growth after a residence of 83 days in the guinea pig eye is shown in Fig. 9. Histologically it is identical with the original avian tumor. Two-day old chicks were inoculated with a transplant which had been in the guinea pig 24 days and within 18 days tumors began to appear. Fig. 10 shows the histological appearance of a growth 107 days after inoculation into the avian host. Grossly and microscopically it was exactly like the original chicken tumor "C." Invasion of the liver by this tumor is seen in Fig. 11. No further chicken passages were made.

Spontaneous chicken tumor "E".—While the chicken in which this tumor was originally observed possessed multiple growths involving various germ layers the growth which has been carried on as Chicken Tumor "E" is a fibrosarcoma (3). This sarcoma is grossly and microscopically similar to the present day Rous type of tumor. It is interesting to note that, contrary to the usual finding, an active filtrate was obtained directly from the original neoplasm. The growth is now in its 17th chicken passage and can be transferred either by cell suspension or filtrate. Fig. 12 shows the cellular structure of the tumor in the 11th chicken transfer. It can be seen that there is a mixture of oval-shaped cells and elongated fibroblasts which are more loosely arranged than in chicken tumor "C." Occasional mitoses are present. The histological appearance of this chicken tumor after 10 days' residence in the rodent eye

DESCRIPTION OF FIGURES 1 TO 6

FIG. 1.—Methylcholanthrene-induced guinea fowl tumor in chick following 9 passages of growth in latter species. Mag. × 95.

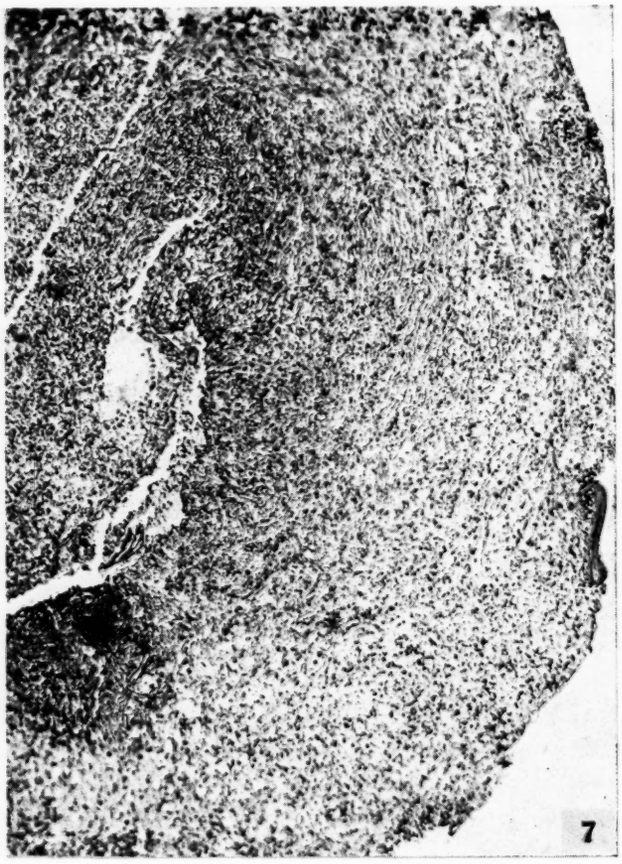
FIG. 2.—Anterior chamber transplant of tumor shown in Fig. 1 after 14 days in guinea pig eye. Mag. × 120.

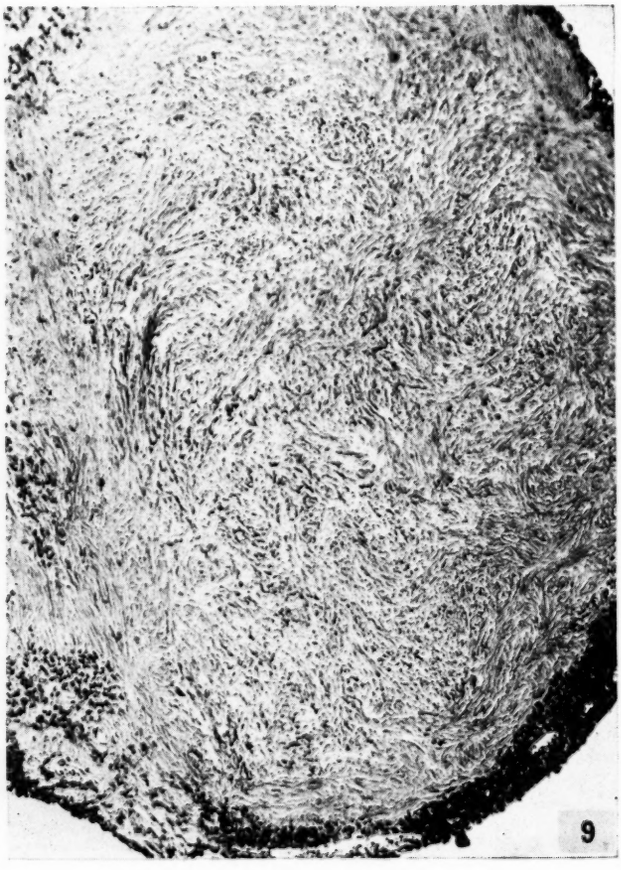
FIG. 3.—First chicken passage tumor following residence of growth 7 days in guinea pig eye. Original tumor may be seen in Fig. 1. Mag. × 120.

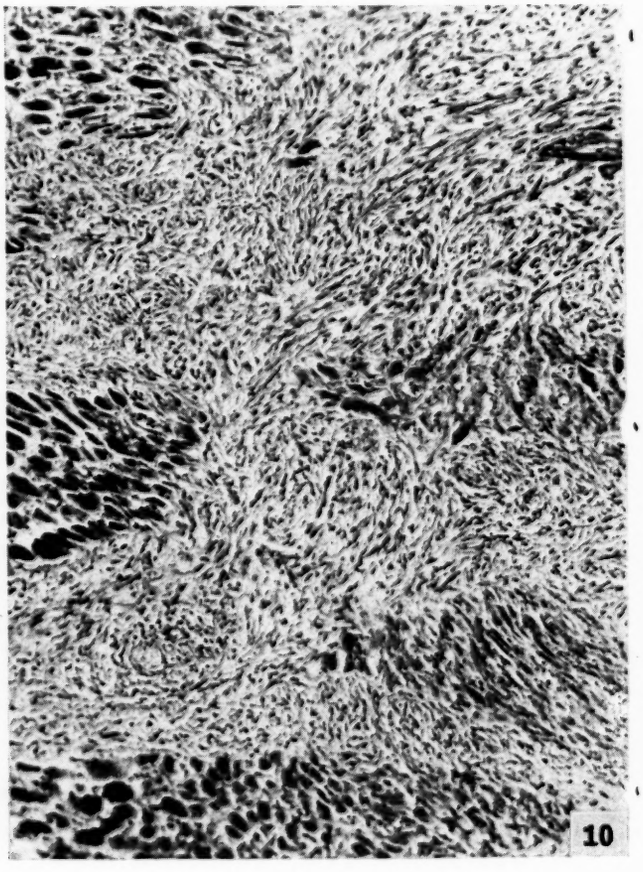
FIG. 4.—Methylcholanthrene-induced pigeon tumor in its fifth passage in this species. Mag. × 120.

FIG. 5.—Anterior chamber transplant of pigeon tumor 19 days after transfer to guinea pig eye. Note presence of mitotic figure. Mag. × 475.

FIG. 6.—Methylcholanthrene-induced chicken tumor in its 26th passage in this species. Mag. × 120.







FIGS. 7-10

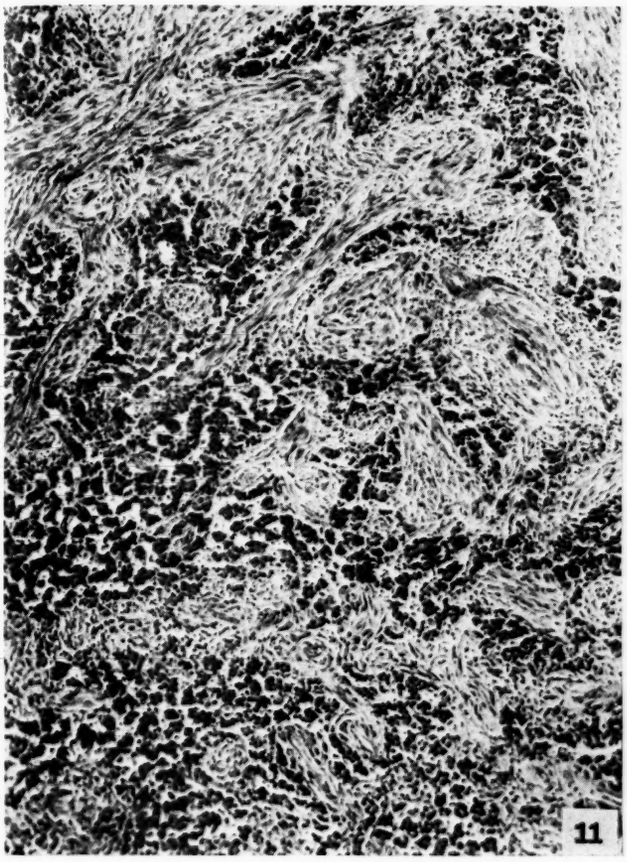


FIG. 11.—Extension of chicken tumor "C" (Fig. 10) to liver of the bird. Mag. × 120.

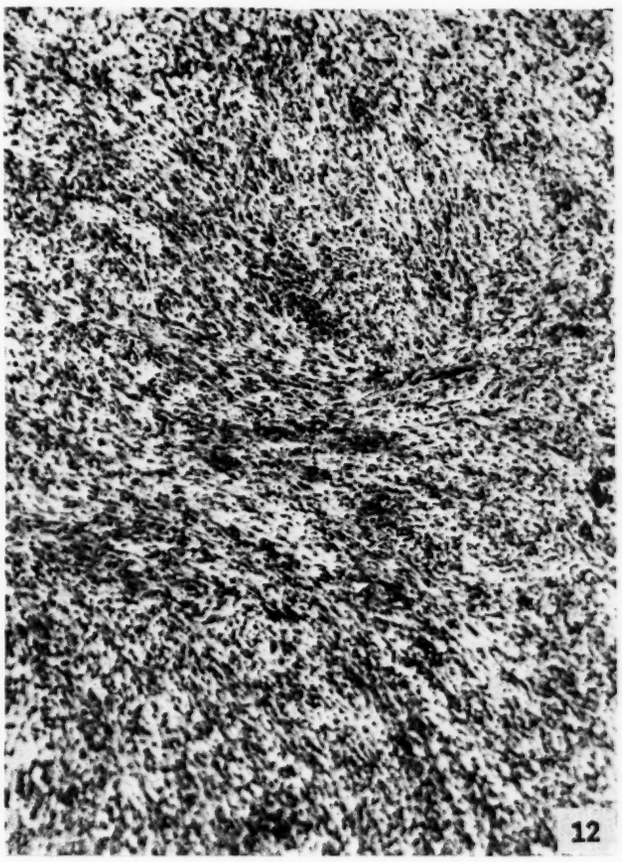


FIG. 12.—Spontaneous chicken tumor "E" in its 11th chicken passage. Mag. × 120.

is represented in Fig. 13. The pattern of "streaming" elongated cells characteristic of the tumor in chickens is largely replaced by oval cells with eosin-staining cytoplasm. The amount of collagen is increased but there are small isolated areas showing hyalin change. The transplant is well vascularized and no mitoses are visible. No transfers of this tissue from the guinea pig were made into chicks.

DISCUSSION AND CONCLUSIONS

It has been found that the Rous sarcoma is not the only avian tumor that will grow in the anterior chamber of the guinea pig eye. The filtrable as well as non-filtrable growths studied here have been shown to possess this ability. Like the Rous sarcoma, the 2 spontaneous and 3 methylcholanthrene-induced tumors discussed did

DESCRIPTION OF FIGURES 7 TO 10

FIG. 7.—Anterior chamber transplant of chicken tumor seen in Fig. 6, 9 days after transfer to guinea pig eye. Mag. × 120.

FIG. 8.—Spontaneous chicken tumor "C" in its seventh passage in chickens. Mag. × 120.

FIG. 9.—Anterior chamber transplant of chicken tumor "C" after 83 days in guinea pig eye. Mag. × 120.

FIG. 10.—Chicken tumor "C" in chicken 107 days after inoculation of transplant from guinea pig's eye. The tissue was in the rodent 24 days. Mag. × 120.

FIG. 13.—The appearance of chicken tumor "E" after residence of 10 days in eye of guinea pig. Mag. × 120.

not grow sufficiently to completely fill the anterior chamber of the rodent eyes as mammalian growths have been found to do (5). The reasons for this difference in behavior must be determined by further study. In general tissue fragments in the alien host grow for 10 to 20 days and then regression becomes evident. However, in the case of one slow-growing spontaneous chicken tumor, healthy sarcoma tissue was recovered from the rodent's eye 83 days after inoculation. Serial passage of these avian tumors in the guinea pig was not attempted nor were efforts made to isolate, during residence of the virus-induced growths in the eye, the tumor virus from various guinea pig tissues as has been possible in the case of the Rous sarcoma (8).

REFERENCES

1. DURAN-REYNALS, F. A Hemorrhagic Disease Occurring in Chicks Inoculated with the Rous and Fuginami Viruses. Yale J. Biol. & Med., **13**:77-98. 1940.
2. DURAN-REYNALS, F., and SHRIGLEY, E. W. Virus Infection as an Etiologic Agent of Cancer. Am. Assoc. Adv. Science Research Conference, Gibson Island, Washington, D. C., 1945, pp. 1-23.
3. DURAN-REYNALS, F., and SHRIGLEY, E. W. A Study of Five Transplantable Chicken Sarcomas Induced by Viruses. Cancer Research, **6**:535-544. 1946.
4. DURAN-REYNALS, F., SHRIGLEY, E. W., and DE HOSTOS, E. Carcinogenesis Induced by Methylcholanthrene in Pigeons, Guinea Fowls, and Ducks. Cancer Research, **5**:11-17. 1945.
5. GREENE, H. S. N. Heterologous Transplantation of Mammalian Tumors. II. The Transfer of Human Tumors to Alien Species. J. Exper. Med., **73**:475-486. 1941.
6. GREENE, H. S. N. The Microscope or the Guinea Pig? Yale J. Biol. & Med., **18**:239-242. 1946.
7. MILFORD, J. J., and DURAN-REYNALS, F. Growth of a Chicken Sarcoma Virus in the Chick Embryo in the Absence of Neoplasia. Cancer Research, **3**:578-584. 1943.
8. SHRIGLEY, E. W. Preliminary Studies on the Recovery of the Rous Sarcoma Virus from Guinea Pig Tissues during Growth of the Tumor in the Anterior Chamber of the Rodent's Eye. Abstract, Am. Assoc. for Cancer Research, Inc., 37th Ann. Meet., Atlantic City, N. J. Cancer Research, **6**:503. 1946.
9. SHRIGLEY, E. W., GREENE, H. S. N., and DURAN-REYNALS, F. Studies on the Variation of the Rous Sarcoma Virus Following Growth of the Tumor in the Anterior Chamber of the Guinea Pig Eye. Cancer Research, **5**:356-364. 1945.

Reciprocal Effects of Natural Immune Bodies from Chickens and Ducks on Variants of a Sarcoma Virus*

F. Duran-Reynals, M.D., and J. W. King

(From the Department of Bacteriology and Immunology, Yale University School of Medicine, New Haven 11, Connecticut)

(Received for publication August 9, 1946)

Like so many other infectious agents, the viruses of chicken sarcomas are far from being static, immutable entities. If coming from tumors grown in chickens of a certain age (6) and injected into newborn or young birds of alien species (4, 5), infection and subsequent adaptation may follow. This process involves a mutation or variation of the causative virus and, as is the case with so many other viruses (9), this variation is manifested by changes in host affinities, tissue affinities, virulence, and type of lesion induced. As a result of this a great variety of stable lines of tumors and leukoses can be obtained from the virus of the Rous sarcoma, and the same seems to be true for other chicken tumor viruses as well (8). Let us point out in passing that these observations answer the objection often raised against the infectious doctrine of cancer that to explain the genesis of the great variety of tumors observed as many viruses would be needed.

The infection of ducks by the virus of the Rous sarcoma has been best studied. Ducks, 24 hours after hatching, were found susceptible to the chicken virus injected intravenously. After this time injections of even large amounts of virus were ineffective, indicating that a state of complete resistance had been rapidly acquired by the aging host (4).

A similar age resistance, but one developing less rapidly and efficiently, was found to take place in chickens injected with some of the duck strains, for such hosts were found still susceptible to the virus for 8 days after hatching, and cell suspensions were sometimes effective in inducing temporary growths in older chickens (4).

On the other hand, chickens are known to develop, by aging, a resistance against virus-induced tumors of their own species, that is, against homologous tumor viruses (2) and the same is true for ducks against homologous tumor viruses (10). These types of resistance are linked with a gradual increase in the blood serum of a neutralizing power for the viruses, and the responsible factors show the general properties of natural antibodies.

The present investigation can be divided in two parts. In the first part we tried to learn whether the resistance of ducks and chickens against chicken and duck tumor viruses, respectively, that is, against heterologous viruses, can be linked with a humoral factor as is the case in the age resistance of the two species against their homologous tumor viruses.

Naturally, the study involved tests in which the sera of ducks and chickens were confronted with heterologous tumor viruses and tested on hosts homologous to those supplying the sera. In these experiments we have attempted to duplicate *in vitro* what can be suspected as operative in the living animal and can therefore be considered as *natural systems*, the same as those in which we studied the effects of the same sera on homologous viruses as tested on homologous hosts (2, 10).

However, other tests can be carried out to study the effect of duck serum on duck and chicken viruses as tested on chickens and the effect of chicken serum on the same two viruses as tested on ducks. These tests do not duplicate any reaction in the living animal and can therefore be considered as *artificial systems*.

The data from both natural and artificial systems are useful from another angle, namely, the study of the changes taking place in the antigenic makeup of the virus in the process of varying from a chicken to a duck tumor virus. This is the subject of the second part of the present publication.

MATERIALS AND METHODS

The chicken tumor employed was the original Rous sarcoma maintained by passage of cell suspensions through Plymouth Rock chicks and young chickens. The duck variants were the closely related strains HV and HC (2) maintained in the same way through young

* Aided by a grant from The Jane Coffin Childs Memorial Fund for Medical Research.

Pekin ducks. Since these strains were lost, some tests had to be carried out with two other duck variants, strains 14(d)7 and 14(d)11, both obtained in this laboratory about one year ago, and similar in virulence and tissue affinities to strains HV and HC.

The virus material used in the tests consisted of extracts of one part of tumor and 19 of saline solution centrifuged for 30 minutes at 3,000 r.p.m.

Nothing in the results suggested that even if a few cells were left in the extracts they were of any account in the tests. Other studies had proved (8) that sera with a neutralizing power for tumor extracts also have a suppressing power on the one hand for cell suspensions and on the other hand for filtrates of the same tumor.

The serum used in each test were always pools of aliquots from the blood of at least five ducks or chickens. The latter were either individuals from 3 to 12 months of age designated as "adults" or from individuals less than 24 hours old designated as "newborn."

The tests were carried out by mixing the serum with an equal volume of tumor extract and letting the mixture stand at 2° to 4° C. for 3 hours. Analogous mixtures were set up employing saline solution instead of serum as controls.

The way these mixtures were injected varied according to whether the virus was homologous to the host into which it was injected (chicken and duck viruses into chickens and ducks, respectively) or heterologous to the host (chicken virus into ducks and duck virus into chickens). In the first sequence, the method was the same as when studying the effect of chicken or duck sera on homologous viruses tested on homologous hosts, (2, 10) that is, adult individuals were injected intradermally in the right side of the breast with the virus-serum mixtures and in the left side, in corresponding locations, with the saline mixtures. Three mixtures containing the tumor extract diluted at 1:20, 1:200 and 1:2,000 were always tested on the same individual. The amounts of mixture injected were 1 or 2 cc. in the case of duck virus and 0.5 cc. in the case of chicken virus.

In the second sequence, in view of the rapid development of age resistance by ducks and chickens against heterologous viruses, the mixtures were tested in newborn individuals. Each of them was injected with one type of mixture containing either serum or saline, the injections being subcutaneous in the breast in the amount of 3 cc.[1]

[1] Even in injecting such young individuals many experiments resulted in failure due to the complete resistance of the hosts injected. In past experiments (4) only 22 per cent of ducklings and 42 per cent of chicks developed tumors when injected with chicken and duck tumor viruses respectively.

Any departure from the general method will be indicated in the appropriate section.

In practically every experiment it was determined by separate tests that the duck and chicken sera used had a strong neutralizing power for the homologous tumor viruses, testing the mixtures on homologous hosts.

TABLE I: EFFECT OF DUCK SERUM ON CHICKEN TUMOR VIRUS TESTED ON DUCKLINGS

	No. of ducklings injected with mixtures of chicken virus plus					
Exper. no.	Serum from newborn ducks	No. with tumors	Serum from adult ducks	No. with tumors	Saline solution	No. with tumors
1	7	7	8	6	8	6
2	5	3	5	0	5	1
3			5	4	5	4
4	4	3	6	0	6	1
5	4	1	6	2	6	2
Total	20	14 (70%)	30	12 (40%)	30	14 (47%)

Effect of duck serum on chicken virus tested on ducklings.—It is clear from the results given in Table I that the serum from newborn as well as from adult ducks was devoid of neutralizing power for the chicken tumor virus when the mixtures were tested on ducklings.

TABLE II: EFFECT OF CHICKEN SERUM ON DUCK TUMOR VIRUS TESTED ON CHICKS

	No. of chicks injected with mixtures of chicken virus plus					
Exper. no.	Serum from newborn chicks	No. with tumors	Serum from adult chickens	No. with tumors	Saline solution	No. with tumors
1	6	0	6	0	3	3
2			6	0	6	6
3*			3	0	3	3
Total	6	0 (0%)	15	0 (0%)	12	12 (100%)

* Serum diluted 1:5.

Effects of chicken serum on duck virus tested on chicks.—The results, summarized in Table II, indicate that the sera from newborn (20 hours old) chicks and also from adult chickens completely neutralized the duck tumor virus testing the mixtures on newborn chicks. The effect of adult chicken serum was the same after dilution at 1:5.

TABLE III: EFFECT OF DUCK SERUM ON CHICKEN TUMOR VIRUS TESTED ON CHICKENS

	No. of areas in the skin of chickens injected with mixtures of chicken virus plus					
Exper. no.	Serum from newborn ducks	No. with tumors	Serum from adu't ducks	No. with tumors	Saline solution	No. with tumors
1	6	3			6	2
2			12	7	12	9
3			24	15	24	19
4			12	12	12	11
5			12	12	12	6
Total	6	3 (50%)	60	46 (76%)	66	47 (71%)

TABLE IV: EFFECT OF DUCK SERUM ON DUCK TUMOR VIRUS TESTED ON CHICKS

Exper. no.	Serum from adult ducks	No. of ducklings injected with mixtures of chicken virus plus No. with tumors	Saline solution	No. with tumors
1	8	7	7	3
2*	7	7		
3	9	9	9	9
Total	16	16 (100%)	9	9 (100%)

* Control animals died accidentally shortly after injection.
In experiments 2 and 3, strains 14(d)7 and 14(d)11 respectively were employed.

Effects of duck serum on chicken virus tested on chickens and of the same serum on duck virus tested on chicks.—Results in Tables III and IV show that duck serum was devoid of a neutralizing effect on both chicken and duck tumor viruses when the mixtures were tested on chickens and chicks respectively.

TABLE V: EFFECT OF CHICKEN SERUM ON DUCK VIRUS TESTED ON DUCKS

Exper. no.	Serum from adult chickens	No. of areas in the skin of ducks injected with mixtures of chicken virus plus No. with tumors	Saline solution	No. with tumors
1	4	0	4	4
2	9	0	9	9
3*	9	0	9	9
4†	6	2	6	6
5†	2	1	2	2
6†	7	4	7	7
7‡	8	4	8	8
Total	45	11 (24%)	45	45 (100%)

* Mixtures incubated at 38° C.
† Serum diluted 1:5.
‡ Serum diluted 1:10.
In experiments 2 and 3, strain 14(d)7 of duck tumor was employed.

TABLE VI: EFFECT OF CHICKEN SERUM ON CHICKEN VIRUS TESTED ON DUCKLINGS

Exper. no.	Serum from adult chickens	No. of ducklings injected with mixtures of chicken virus plus No. with tumors	Saline solution	No. with tumors
1*	4	0	4	4
2*	4	0	4	4
3*	3	1	3	2
4	3	1	3	2
Total	14	2 (14%)	14	12 (85%)

* Mixtures incubated at 38° C.

Effects of chicken serum on duck virus tested on ducks and of the same serum on chicken virus tested on ducklings.—It is evident from the results presented in Tables V and VI that chicken serum had a strong neutralizing effect on both chicken and duck viruses testing the mixtures on ducks and ducklings respectively. It will be noted in Table V that dilution of the serum somewhat decreased its neutralizing power, and in Tables V and VI that neutralization takes place just as well at 38° C. because experiments 2 and 3 of Table V and 1 and 2 of Table VI were carried out simultaneously using the same materials. The latter tests also show that the virus of another duck tumor, strain 14(d)7, was also neutralized by the chicken serum.

DISCUSSION

Analysis of the results will be facilitated by listing the 8 possible sequences that can be developed by combining the 3 factors at play, namely, (a) serum containing the immune bodies; (b) tumor viruses with which the serum is confronted; (c) and the host in which the effect is tested. This has been done in Table VII. It will be noted that the first 4 sequences

TABLE VII: A SUMMARY OF THE TESTS CARRIED OUT COMBINING SERA, TUMOR, VIRUSES AND HOSTS EMPLOYED

Sequences	Sera	Tumor virus	Host on which the effect was tested	Neutralization of the virus	
1	Duck	Duck	Duck	+	Natural systems
2	Duck	Chicken	Duckling	−	
3	Chicken	Chicken	Chicken	+	
4	Chicken	Duck	Chick	+	
5	Duck	Duck	Chick	−	Artificial systems
6	Duck	Chicken	Chicken	−	
7	Chicken	Duck	Duck	+	
8	Chicken	Chicken	Duckling	+	

are *natural systems* in which serum and host belong to the same species, and the last 4 sequences are *artificial systems* in which serum and host belong to different species. We can now proceed to discuss the results of the first part of our investigation as follows.

Since the serum of ducks is devoid of any effect on chicken tumor viruses as tested on ducklings (sequence 2) but neutralizes duck tumor viruses as tested on ducks (sequence 1) it would seem that the resistance of ducks is entirely different whether it is directed against the heterologous chicken virus or against the virus after it has varied and has become a homologous duck tumor virus. In the latter case, the resistance can be linked with the presence of immune bodies naturally present in the blood, but in the former case the resistance seems to fall in the field of the so-called *species resistance* and, within this field, it should be classed with those cases in which the refractory state is not installed till birth, the embryo being susceptible (*e.g.* herpes virus in chicken embryo) rather than with those other cases in which the refractory state is apparently absolute from embryonic life (*e.g.* virus III in chick embryo), the cells failing entirely to support the growth of the virus. This distinction is of the utmost importance to our problem because it is only within a very short period after hatching—and presumably during embryonic life—that a chicken virus can be inoculated with the prospect that if the virus varies and adapts itself to the new host later in adult life a neoplasm looking

to all intents and purposes like a spontaneous neoplasm may develop.[2]

The situation is different in the case of chickens for in these animals the resistance against both homologous and heterologous viruses (sequences 3 and 4) seems to be of the same sort, namely humoral (2, 3, 8, 10).

It will be noted, though, that as is the case with so many other infections, the resistance of a humoral type is far less effective than is species resistance. The latter type of resistance in the case of ducks against chicken tumor viruses is *absolute*, one that withstands the inoculation of enormous amounts of virus (*e.g.* 20 cc. of a thick suspension of tumor cells) (2, 8). On the other hand, the age resistance of chickens and ducks against their homologous tumor viruses (2, 10) is often of a relative type and does not become pronounced, as when tumors have regressed after an initial phase of growth. To a certain extent the same is true for the resistance of chickens against the heterologous duck virus because in this case chicks 8 days old could still be infected with filtrates of duck tumor strains HV and HC,[3] and cell suspensions of these tumors were sometimes effective in inducing temporary growth in older chickens (2). Although the term "heterologous" is being applied to duck tumor viruses in relation to chickens, it is clear that the degree of heterogeneity is far less pronounced than in the case of chicken tumor viruses in relation to ducks.

At any rate it is interesting to consider in the 4 sequences so far analyzed that the presence of natural immune bodies in a given host is an indication of actual or potential susceptibility to the antigen virus. Although wholly unexplained, the reason why these antibodies exist is most puzzling, especially as they are already present 24 hours after hatching and as tumors and leukoses have never been observed as occurring spontaneously even in old individuals of the Pekin ducks employed.

The results from the last 4 sequences integrating the artificial systems hardly deserve comment; they show that the sera of ducks exert no effect on duck or chicken tumor viruses as tested on chicks or chickens (sequences 5 and 6) but that the chicken serum neutralizes the same viruses as tested on ducks or ducklings (sequences 7 and 8).

Analyses of the 8 sequences furnish us with the necessary data to discuss the second part of our investigation, namely, the antigenic changes taking place in the virus in the process of varying from a chicken virus into a duck virus. The results can be summarized by the following statement: *The natural immune body of chickens neutralizes both chicken and duck tumor viruses in both chickens and ducks, whereas the natural immune body of ducks neutralizes only the duck tumor virus in ducks.* This situation suggests a hierarchy, as it were, in the quality of the viruses and of their respective antibodies.

One could visualize that in the process of variation and subsequent adaptation from chicken to ducks, the virus has acquired new receptors which are still acted upon by the chicken antibody whereas the antibody against the duck variant is active only on these receptors, while it is inactive on the receptors of the original chicken virus. Even if the newly acquired receptors of the duck variant are neutralized by the duck antibody, the virus will still retain the capacity to infect chicken cells, as sequence 5 shows, because the chicken receptors are still present. However, one must assume that the latter receptors have been changed somewhat in the sense that either the virus has lost much of its former affinity for chicken cells or has become more vulnerable to the natural chicken immune bodies because the infective power for chickens of the duck variant is far less than it was before the virus varied.[4]

It seems superfluous to add that the problems here discussed are identical with those of the antigenic changes involved in virus variation in general. The cases of the viruses of vaccinia, variola, and alastrim and still others of the pock disease group (9, 12) and also those of several plant diseases (1, 11) are especially pertinent.[5]

SUMMARY

The immune bodies that develop naturally in the blood of aging chickens neutralize the viruses of both the

[2] One could here speculate, as we have done in another publication, (7) on the causes of this species resistance, an extremely important yet almost unexplored field; whether it is due to an inherent lack of affinity between virus and cell as in the case of bacteriophage and non-susceptible bacteria, or to an impossibility, either inherent or acquired by aging, of the cell to support growth of the virus, or to other causes.

[3] Let us note in this connection that cells and viruses from duck strains 14(d)7 and 14(d)11 are more active for older chickens than are strains HV and HC.

[4] It is interesting to note that judging by the experiment of Table II the serum of newborn chicks had a neutralizing power for the duck virus as tested on chicks, whereas, as shown elsewhere (2), the same sera neutralized chicken tumor virus on chicks only occasionally.

[5] Several experiments on active reciprocal protection have been carried out by treating ducks with chicken tumor virus and vice versa. The loss of duck strains HV and HC prevented completing the studies but the indications were that there was some degree of reciprocal protection. The same was true in the case of chickens in which a duck tumor grew and regressed, since about 35 per cent of them were found resistant to the further inoculation of extracts or cell suspensions of the Rous sarcoma.

chicken tumor (Rous sarcoma) and its duck variants as tested on both chickens and ducks. Therefore, the resistance of chickens against these viruses is linked with the presence of humoral factors and might be explained on this basis.

On the contrary, comparable immune bodies from aging ducks neutralize only duck tumor viruses as tested on ducks, but not as tested on chickens; nor do they neutralize chicken tumor viruses as tested on either chickens or ducks. Therefore, the refractory state of ducks against heterologous chicken viruses, solidly installed very shortly after hatching, cannot be linked with humoral factors but rather belongs to the so-called species resistance. However, the resistance of these hosts against the virus after it has varied and has become a duck virus is linked with humoral factors and might be explained on this basis. One may add that, with the tumor viruses here studied, the species resistance is far more effective than the humoral resistance, and that the presence of natural specific immune bodies against the viruses is an indication of actual or potential susceptibility to these viruses.

Analysis of the 8 possible sequences that can be obtained by testing the effect of chicken and duck sera against homologous and heterologous tumor viruses on homologous and heterologous hosts indicates that a pronounced antigenic change has taken place in the virus in the process of variation, and tentatively one may form an idea of the nature of this change.

REFERENCES

1. Bawden, F. C. Plant Viruses and Virus Diseases. Second edition. Waltham, Mass.: Chronica Botanica Company. 1943.
2. Duran-Reynals, F. Neutralization of Tumor Viruses by the Blood of Normal Fowls of Different Ages. Yale J. Biol. & Med, **13**:61-76. 1940.
3. Duran-Reynals, F., and Estrada, E. Protection of Chick Against Rous Sarcoma Virus by Serum from Adult Chicken. Proc. Soc. Exper. Biol. & Med., **45**:367-372. 1940.
4. Duran-Reynals, F. The Reciprocal Infection of Ducks and Chickens with Tumor-Inducing Viruses. Cancer Research, **2**:343-369. 1942.
5. Duran-Reynals, F. The Infection of Turkeys and Guinea Fowls by the Rous Sarcoma Virus and the Accompanying Variations of the Virus. Cancer Research, **3**:569-577. 1943.
6. Duran-Reynals, F. The Age Factor in the Adaptability of a Sarcoma Virus to Other Animal Species. Science. In press.
7. Duran-Reynals, F. Age and Infection. J. of Gerontology, **1**:358-373. 1946.
8. Duran-Reynals, F. Unpublished observations.
9. Findlay, G. M. Variations in Animal Viruses. A Review. J. Roy. Micr. Soc., **56**:213-299. 1936.
10. King, J. W., and Duran-Reynals, F. Development by Ducks of Natural Neutralizing Antibodies for a Duck Variant of the Rous Sarcoma Virus. Yale J. Biol. & Med., **16**:53-57. 1943.
11. Kleczkowski, A. Quantitative Studies on the Serological Reactions of Some Plant Viruses and of a Pea Nodule Bacterium (*Rhizobium leguminosarum*). Brit. J. Exper. Path., **22**:44-58. 1941.
12. Van Rooyen, C. E., and Rhodes, A. J. Virus Diseases of Man. London: Oxford University Press. 1940.

On the Production of Sarcoma with Wheat Germ Oil*

Paul N. Harris, M.D.

(*From the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis 6, Indiana*)

(Received for publication July 24, 1946)

Following the announcement by Rowntree, Lansbury, and Steinberg (15) of the development of sarcoma within the peritoneal cavity of albino rats to which ether-extracted wheat germ oil had been fed, and the simultaneous appearance of a confirmatory publication by Dorrance and Ciccone (10), various investigators (1, 4, 5, 7, 9, 11-14, 21, 22) attempted to produce tumors in this manner, but to no avail. Nevertheless, in later publications by Rowntree and his associates (16-19) the number of rats that developed tumors increased with the passage of time, and ultimately a total of 109 rats was found to have developed tumors (19). Blumberg (4) suggested that the failure of others to confirm the work of Rowntree and his associates was due to their not having closely reproduced the conditions prevailing in the experiments at the Philadelphia Institute for Medical Research, and stressed the importance of using the same type of diet, the same strain of rats, and the same kind of oil as was used by Rowntree and his group. It is evident that at least one additional unknown factor exists, since the percentage tumor incidence in Rowntree's laboratory after having reached an impressive level began to fall and steadily diminished to zero (20).

Investigation was begun in this laboratory early in 1938 in an attempt to confirm Rowntree's observations and to ascertain the nature of the carcinogen concerned. We had the advantage of having the close cooperation of Rowntree and his co-workers, and in addition to having had the opportunity of personally going over their material and methods, we were generously supplied with rats, diet, and oil from their laboratory.

METHODS

At first we prepared our oil by ether extraction in a percolator, but later prepared it by 24 to 48 hours continuous ether extraction in a Soxhlet apparatus (a method recommended to us by W. R. Brown and used by him in Rowntree's laboratory). Some of our oil was prepared from wheat germ obtained from a local mill, but the major portion was prepared from wheat germ obtained from the same source as was that used by Rowntree and his associates; that is, General Mills, Minneapolis. The ether-extracted oil contained a large amount of greenish sediment and some flour.

The amount of flour in the oil was kept to a minimum by pouring ether over the wheat germ in the extractor, and allowing it to drain off rapidly, thereby carrying off much of the loose flour. The flour was removed by filtration and the rinsing repeated. After a second filtration the ether was used for extraction of the oil. After the extraction the ether was eliminated from the oil by vacuum distillation. Spectroscopic examination of the oil and sediment revealed no substances giving a spectrum like that of the better known carcinogenic hydrocarbons.

Feeding of oil.—Oil was administered to some animals by admixture with food of 2 to 5 cc. of oil per rat per day. With the larger doses of oil it not infrequently occurred that the rats failed to consume all their food and oil accumulated. At such times addition of more oil was omitted for a day or two. Other rats received 2 to 8 cc. of oil by *gavage* 5 times per week. Still others received 2 to 8 cc. of oil every day, at times by *gavage* and at times on the food.

The greater number of rats used were obtained from local breeders, and were descended from the Wistar strain.

Several variations in diet were made. The greater number of rats received our colony rat diet consisting of:

Ground yellow corn	53 parts
Linseed oil meal	4 "
Meat scraps	13 "
Soy bean meal	5 "
Wheat middlings	10 "
Wheat bran	8 "
Casein	3 "
Brewer's yeast	2 "
Mineral mixture	2 "
Cod liver oil	1 part

The mineral mixture consisted of:

Calcium carbonate	4587 parts
Bone meal	4588 "
Ferric citrate	300 "
Iodized salt	500 "
Potassium iodide	25 "

* Presented at the 34th Annual Meeting of the American Association for Cancer Research, Chicago, Illinois, April 16, 1941.

With the purpose of ascertaining whether the lower fatty acids would have an inhibitory effect on tumor development, some animals received a diet consisting of:

	per cent
Ground yellow corn	34
Ground whole wheat	34
Wilson's vacuum dried liver	2
Brewer's yeast, Anheuser Busch Strain K	2
Linseed oil meal	2
Valentine's meat residue	14
Cod liver oil	1
Calcium carbonate	0.5
Sodium chloride	0.5
Butter-fat	10

For comparison, other animals were given an identical diet except that the butter was replaced by lard. Animals on both these diets each received about 5 gm. of fresh kale twice a week. (In Table II these are labeled butter and lard.) Still other rats received food supplied by Rowntree.

Eighty-five rats survived treatment for 3 months or more. None of these animals developed a tumor, although 12 received a total of 800 to 1000 cc. of oil each, and 25 received more than 1000 cc. each. See Table I for a summary of duration of treatment and of

TABLE I: ORAL ADMINISTRATION OF WHEAT GERM OIL

Number of Rats	Duration of treatment, Mo.	Total dose of oil per rat cc.	Rats surviving after oil exhausted	Survival period after oil exhausted, Mo.
4	3-4	220-380	0	—
14	4-5	210-430	11	5-18
9	5-6	330-730	6	4-14
4	6-7	440-870	2	8-14
10	7-8	590-980	5	2-16
5	8-9	680-860	0	—
15	9-10	410-1,050	13	1-11
5	10-11	1,050-1,250	2	6-12
4	11-12	1,140-1,330	2	2½-3
1	12-13	1,250	0	—
4	13-14	1,280-1,610	0	—
2	14-15	1,410-1,560	1	12
3	15-16	1,450-1,610	2	3-11
1	16 17	1,540	0	—
4	17-18	1,350-1,780	3	4-8
Total 85			47	

total dose given, and Table II for variations made in strain of rats, type of diet, and type of oil.

Since it was thought that the diet used in Rowntree's laboratory was low in vitamins of the B complex and that this might be an important factor, one group of rats was placed on a diet mildly deficient in thiamine, a second on a diet very deficient in thiamine, a third on a diet mildly deficient in riboflavin, and a fourth on a diet very deficient in riboflavin. The combination of oil feeding and vitamin deficiency was so detrimental, that in order to preserve the animals' lives it became necessary at intervals to give them an adequate diet for several days. In this variation 39 rats were used and they survived from 1 week to 10 months without developing tumors. In this experiment our own oil, as well as that supplied by Rowntree, was used.

Injection of oil.—Early in our conversations with Rowntree and his associates we had been informed that tumors could be produced by injecting the oil. Although we were primarily interested in producing tumors by feeding, we began injection of oil into small numbers of rats and mice. Preliminary results were

TABLE II: VARIATIONS IN STRAIN OF RATS, TYPE OF DIET, AND TYPE OF OIL

Number of Rats	Strain	Diet	Oil
8	Philadelphia*	Philadelphia	Philadelphia
25	"	"	Lilly
11	Lilly	Lilly (colony)	Philadelphia
4	"	Philadelphia	Lilly
31	"	Lilly (colony)	"
6	"	Butter and Lard	"
Total 85			

* The rats, diet, and oil denoted by "Philadelphia" came from Dr. Rowntree's laboratory at the Philadelphia Institute for Medical Research.

mentioned in discussion of the papers presented by Rowntree and his group at the Richmond meeting of the American Association for Cancer Research in 1939, and later results were presented at the Pittsburgh meeting of the Association in 1940. At this time tumors had been obtained in 2 mice and 8 rats. The incidence of tumors in rats was impressive, 25 per cent of the animals that lived 9 months or more after treatment was begun ultimately developed sarcoma. Rats received from 0.5 to 1 cc. subcutaneously or intraperitoneally, with repetition of the dose at weekly intervals if the animals appeared able to tolerate it. Some of these animals received a clear oil obtained by allowing the turbid oil to settle and decanting the supernatant portion, but the greater number received the turbid oil. In rats, tumors had developed only as a consequence of intraperitoneal injection of turbid oil, but one of the mouse tumors developed after subcutaneous, and the other after intraperitoneal injection of oil. The mice received from 0.1 to 1 cc. with repetition as often as seemed possible.

In view of our inability to obtain tumors by feeding the oil, it was decided in 1939 to investigate in more detail the induction of tumors by injection of oil. For reasons which seemed valid at the time, and with the expectation of increasing the yield of tumors, the technic was altered. Since no tumors had yet developed following subcutaneous injection of oil into rats, this method of administration was abandoned, although an

additional 24 mice received subcutaneous injections. In view of the fact that no tumors had yet appeared in animals injected with clear oil, it seemed that the carcinogenic activity lay in the sediment, and that a more potent product would be obtained by concentrating the sediment. This was accomplished by allowing the oil to settle for 3 weeks or more and decanting the upper half. This "concentrated" oil was used for intraperitoneal injection into rats and for subcutaneous and intraperitoneal injection into mice, but contrary to expectation, no tumors were obtained thereby. As controls for the injection of sediment-containing oil, 1 group of rats received expressed oil, produced commercially for vitamin E therapy, a second group was given the supernatant oil obtained in "concentrating" the sediment-containing oil, and a third group received a mixture of this supernatant oil and a large amount of flour. The essential data are presented in Tables III, IV, and V.

TABLE III: RESULTS OF SUBCUTANEOUS (SC) AND INTRAPERITONEAL (IP) INJECTION INTO MICE OF ETHER-EXTRACTED WHEAT GERM OIL

Type of oil	Given	Number of Mice	Mice surviving after 9 mo.	12 mo.	18 mo.	Tumors
Supernatant	IP	30	0	0	0	0
Whole and "Concentrated"	IP	232	12	4	0	1
Second Extraction	IP	36	0	0	0	0
Whole and "Concentrated"	SC	48	28	14	3	1
Second Extraction	SC	24	16	7	0	0
Total		370	56	25	3	2

In order to ascertain whether or not all the carcinogenic material had been removed from the wheat germ by one extraction, the germ was subjected to a second extraction lasting 120 hours. A small amount of highly toxic oil was obtained by this method (listed in Tables III and IV as Second Extraction oil). Small doses were administered to mice and rats, but no tumors developed. Since the rats received a total of only 1.5 to 6.7 cc. it cannot be said that this oil was incapable of producing tumors, but it is evident that it was not highly carcinogenic.

Mice proved to be unsatisfactory for this experiment. Except with very small doses, subcutaneous injections were usually followed by ulceration and loss of inoculum. The mortality rate following intraperitoneal injection was very high, and not many injections could be repeated. Although 370 mice were injected with different types of wheat germ oil, only 2 developed tumors, 1 after subcutaneous (No. 60), and 1 after intraperitoneal (No. 8) injection (see Table III).

TABLE IV: RESULTS OF INJECTION OF OIL INTO RATS

Type of oil	Given	Number of rats	Rats surviving after 9 mo.	12 mo.	18 mo.	Number with tumors	Latent period, mo.	No. given 20 cc or more
Turbid	IP	85	40	22	6	10	9-25	29
"Concentrated"	IP	91	51	47	18	0	—	38
Supernatant	IP	67	40	22	9	3	12-14	33
Supernatant plus flour	IP	42	27	22	5	1	20	19
Expressed	IP	30	19	15	2	1	17	16
Second Extraction	IP	25	16	15	5	0	—	—
Turbid	SC	12	9	9	3	0	—	12
Supernatant	SC	8	6	5	2	1	19	8
Total		360	208	157	50	16	—	155

Mouse No. 8 received five 0.5 cc. doses at weekly intervals and died 8 months after treatment was begun. The peritoneal cavity was obliterated by fibrous adhesions. Below the costal margin, anterior to the intestines, and adherent to the anterior abdominal wall was a firm mass measuring 3.8 × 2.8 × 2.2 cm. (Fig. 5). There was a central cyst containing pus and mealy, yellowish brown material. Microscopically the tumor was uniform and consisted of bundles of spindle cells with not much collagen (Fig. 9). Mitoses were present but not abundant. In places oil globules were seen well within the tumor.

Mouse No. 60 received two 1 cc. doses of oil at an

DESCRIPTION OF FIGURES 1 TO 10

FIG. 1.—Tumor from rat No. 95. The superior margin is ulcerated and necrotic. A portion of abdominal wall with skin and hair is attached to each side. Mag. × 1.06.

FIG. 2.—Tumor from rat No. 224. Tumor tissue lies to right and a cyst to left. Mag. × 1.06.

FIG. 3.—Tumor from rat No. 255. This tumor was transplanted and carried through 77 generations. Mag. × 1.06.

FIG. 4.—Tumor from rat No. 256. Small cysts are seen throughout. Mag. × 1.06.

FIG. 5.—Tumor from mouse No. 8. There is large central cyst.

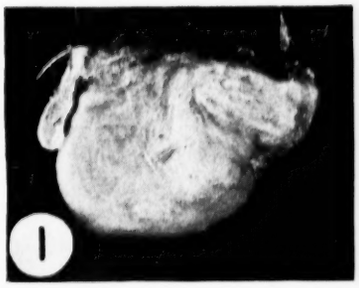
FIG. 6.—Tumor from mouse No. 60. Mag. × 1.03.

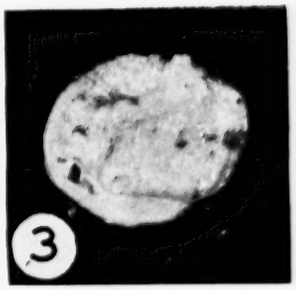
FIG. 7.—Tumor from rat No. 220. The cystic character of the mass with a nodule of tumor in the wall is well shown. Mag. × 1.03.

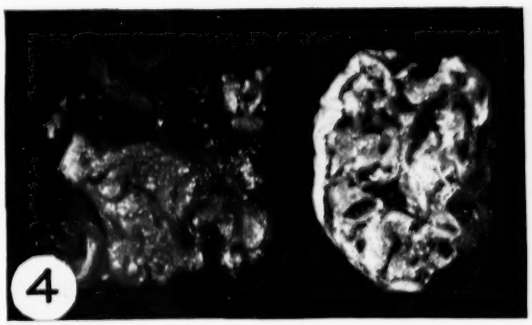
FIG. 8—Tumor from rat No. 264. Cystic cavity is present on each side. Mag. × 1.03.

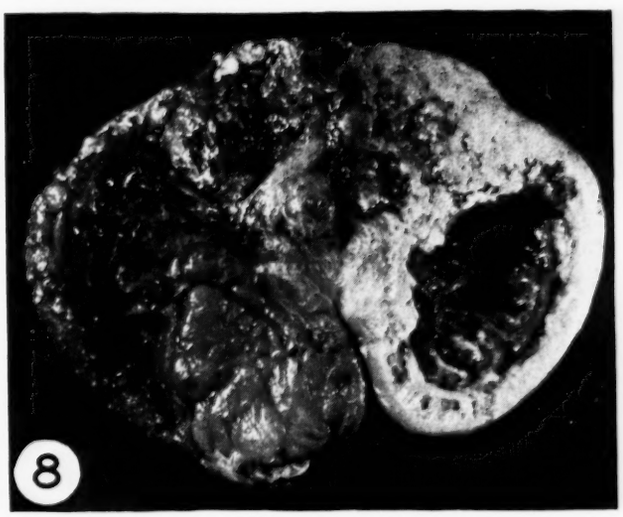
FIG. 9.—Tumor from mouse No. 8. The cells are uniform in size and shape and orderly in arrangement. Hematoxylin and eosin. Mag. × 120.

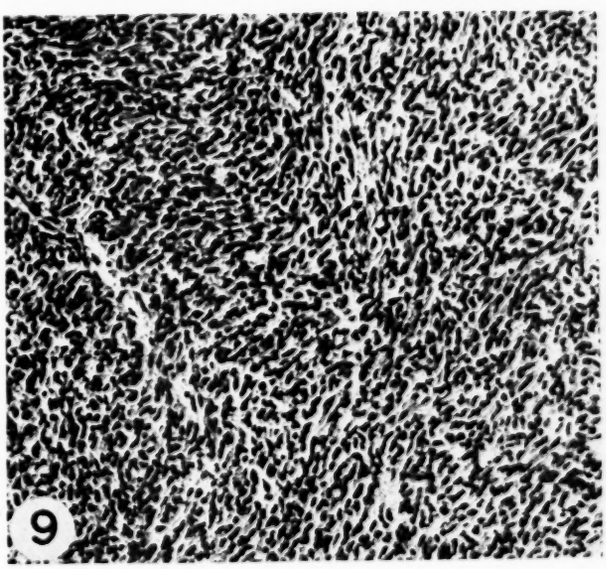
FIG. 10.—Tumor from rat No. 95. There is an abundance of collagen, but rapid growth is evidenced by 3 mitoses lying nearly in line with a diagonal connecting the right upper and lower left corners. Hematoxylin and eosin. Mag. × 120.

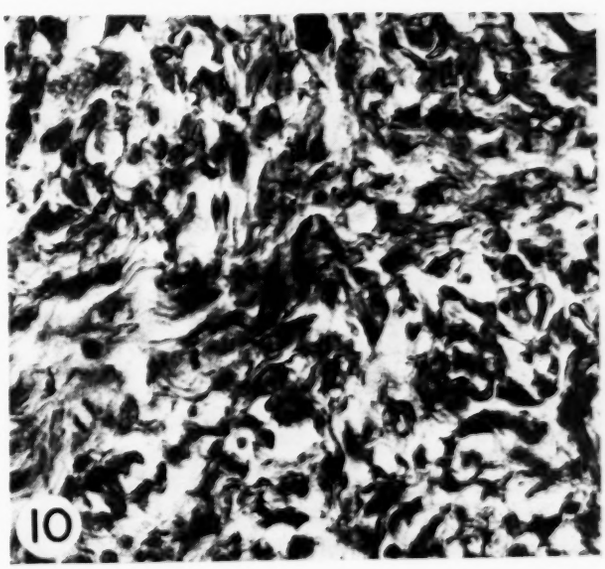




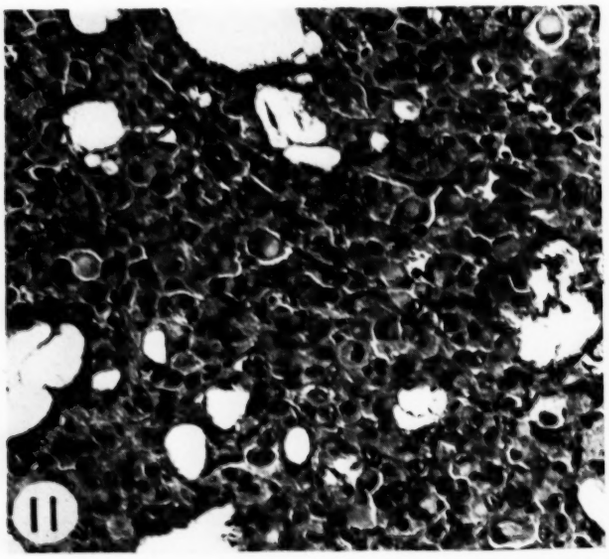


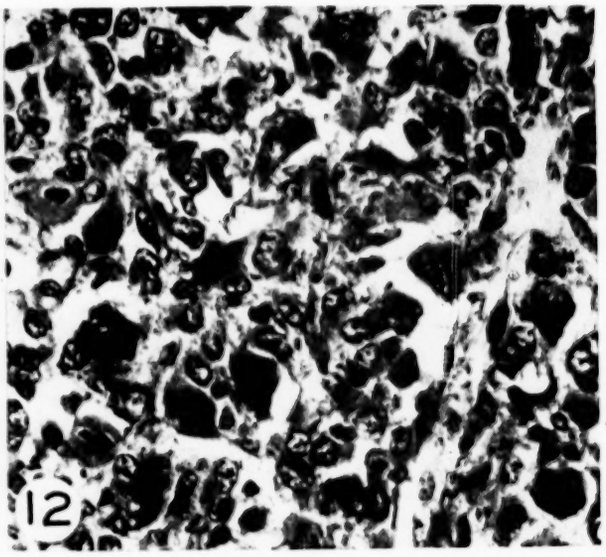


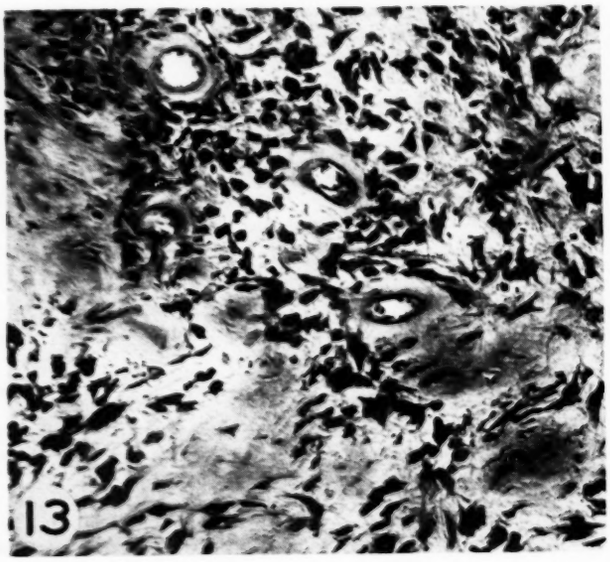


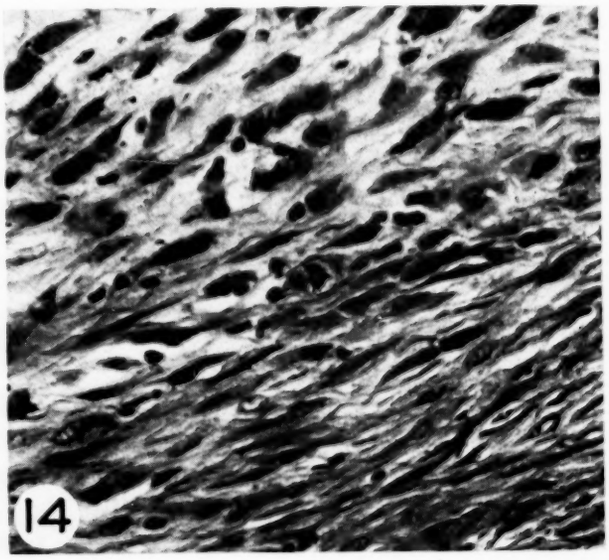


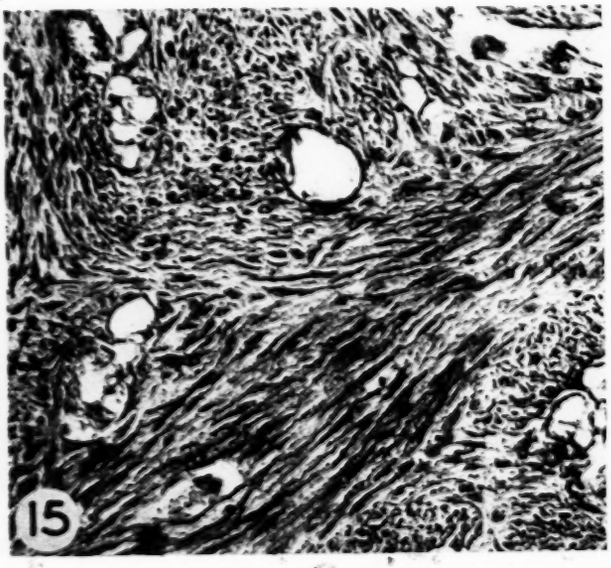
FIGS. 1-10

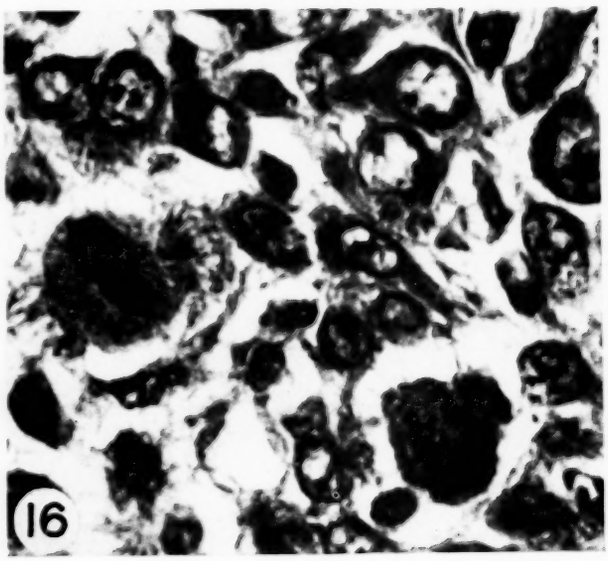












FIGS. 11-16

interval of 1 month. One year later it died with a tumor measuring 3.3 × 2.7 × 2 cm. and lying at the site of injection (Fig. 6). Microscopically it, too, was uniform, and consisted of bundles of fusiform cells with few mitoses and a moderate amount of collagen. In places oil globules were seen well within the tumor.

Rats tolerated injection of oil much better than did mice, but occasionally, following subcutaneous injection, necrosis of the skin occurred and the inoculum was lost. Intraperitoneal injection was followed in a few weeks by obliteration of the peritoneal cavity by fibrous adhesions. The rapidity with which this response occurred depended upon the nature of the oil and the amount of sediment.

Very few or no adhesions were found in rats that received injections of expressed oil. There were, however, many thin yellow plaques ranging up to 4 or 5 mm. in diameter, most numerous on the diaphragm, but found on all peritoneal surfaces. These plaques consisted of oil-laden cells embedded in a small amount of collagenous tissue. Although the animals received from 6 to 30 cc. of oil, little or no free oil was found at postmortem examination. The peritoneal cavity of 1 rat (No. 599) contained about 50 cc. of bloody fluid. There were many thin yellow plaques and the peritoneum was greatly thickened, this being particularly noticeable in the omentum and mesentery. The spleen was encased in a layer of gray tissue nearly 3 mm. thick, and there were many ellipsoidal nodules on the mesentery. The diaphragm and parietal pericardium were thickened by similar tissue. Some hemorrhage had occurred in the mesentery. Microscopically this thickening was seen to be due to a low grade fibrosarcoma composed of fusiform cells with few mitoses and a considerable amount of collagen.

The supernatant ether-extracted oil was more irritating than expressed oil, and intraperitoneal injection ultimately led to obliteration of the peritoneal cavity, but less quickly than did injection of sediment-containing oil. After several months of treatment, a layer of tough, yellowish brown tissue of variable thickness and extent was found in the peritoneal cavity. This consisted of collagenous tissue in which were seen oil globules surrounded by foreign body giant cells, and many large phagocytes laden with oil. In addition, one or more small encapsulated collections of oil or pus, or both oil and pus, were usually seen. Some collections of grumous, brown oily material were also found. Quite often, animals that had been untreated for several months before death showed less extensive adhesions, doubtless because of resorption. In some instances, following injection, large intraperitoneal abscesses formed and ruptured through the abdominal wall. When this happened, treatment was discontinued until recovery had occurred.

For the sake of brevity, only a general description of the tumors obtained as a result of intraperitoneal injection of the oil will be given. Most of the tumors lay between the intestines and the anterior abdominal wall; they were surrounded by the previously mentioned tough, brown, collagenous tissue, but were sharply demarcated and easily dissected free. One rat (No. 95) had a tumor measuring 2.8 × 2.7 × 1.5 cm. attached to the abdominal wall about a draining sinus; this tumor evidently originated in the wall of an abscess (Fig. 1). Three others (Nos. 72, 205, and 546) were undoubtedly of similar origin. Rat No. 546 had an encapsulated abscess 2.5 cm. in diameter containing turbid oil and glairy fluid; at one end there was a sarcomatous plaque 3 mm. thick and 1 cm. in diameter. Rat No. 205 had a larger abscess with, at one end, a layer of tumor tissue 3 cm. broad and 1 to 5 mm. thick. Rat No. 72 had a large encapsulated abscess, at one end of which was a sarcomatous plaque 3 mm. thick and 15 mm. in diameter. Immediately adjacent was a soft tumor measuring 1 × 1.5 × 2 cm.

Rat No. 264 had a tumor measuring 7 × 5.5 × 4 cm. and containing 2 cysts filled with glairy fluid. The wall of one cyst was of irregular thickness, and the wall of the other was uniformly 3 mm. thick (Fig. 8). Rat No. 220 had a cyst measuring 6.8 × 5 × 3 cm. that contained 25 cc. of mucinous fluid (60 cc. had been aspirated 5 days earlier). The wall was 2 to 3 mm. thick, but at one point a tumor measuring 2.5 × 2 ×

DESCRIPTION OF FIGURES 11 TO 16

Sections stained with phosphotungetic acid and eosin

FIG. 11.—Tumor from rat No. 205. Several globules of injected oil are included. The tumor cells are large, and rounded or polyhedral. Several mitoses are included in the field, but not all can be readily distinguished. Mag. × 120.

FIG. 12.—Tumor from rat No. 256. This region is anaplastic and rapidly growing, but the greater portion of the tumor is more typically fibrosarcomatous. Mag. × 240.

FIG. 13.—Tumor from rat No. 224. Collagen is abundant in this region. Mag. × 120.

FIG. 14.—Tumor from rat No. 224. In the lower half of the field the cells are thin and collagen is fairly abundant, and in the upper half the cells are larger and the intercellular substance is more loose. Mag. × 240.

FIG. 15.—Tumor from rat No. 264. This is a well differentiated fibrosarcoma with several masses of injected oil. Mag. × 120.

FIG. 16.—Tumor from rat 264. This is a less well differentiated region and includes 2 mitoses. Mag. × 480.

1.2 cm. projected into the cyst (Fig. 7). Two other rats had tumors with large cystic regions. Rat No. 224 had a mass measuring 4 × 2.5 × 2.3 cm. with a tumor measuring 2.5 × 1.5 × 1.2 cm.; the rest of the mass was occupied by serous fluid containing brown, flaky material (Fig. 2). Rat No. 256 had a mass measuring 5 × 4 × 2 cm. and incorporating a tumor measuring 3.5 × 3.5 × 2 cm., the rest of the mass consisting of grumous brown material (Fig. 4).

The other 6 rats that received the oil intraperitoneally had solid tumors ranging in diameter from 2 cm. to 5.7 × 5 × 3 cm. The larger tumors had undergone much necrosis, and some contained cystic foci. Some were soft and others were moderately firm. One rat (No. 429) had pulmonary metastases. One tumor (No. 255) was transplanted and was carried through 77 generations before being dropped.

Rat No. 155 received the oil subcutaneously, and ultimately developed a tumor; after the growth had reached a size of 4 × 5 × 6 cm., it was removed and successfully transplanted. It was abandoned after 17 generations.

As would be expected, the tumors varied greatly in microscopic appearance (Figs. 10 to 16). Some contained much collagen and were composed of spindle-shaped cells of uniform size and appearance arranged in interlacing bundles and whorls. Others were more pleomorphic, with many cells of irregular size and shape, numerous giant cells, abundant mitoses, and less collagen. One tumor (No. 205) was extremely anaplastic, consisting of large, rounded, or polyhedral cells. This was presumed to be a mesothelioma (Fig. 11). The others were fibrosarcomas. In many tumors, globules of the injected oil were seen.

TABLE V: RATS DEVELOPING TUMORS AS A RESULT OF INJECTION OF WHEAT GERM OIL

Rat number	Type of oil	Given	Total dose cc.	Duration of treatment, mo.	Latent period, mo.
95	Turbid	IP	14	7	9
205	Turbid	IP	21	9	10
661	Turbid	IP	33	10	11.5
264	Turbid	IP	23	6.5	12
220	Turbid	IP	30	6.5	12
224	Turbid	IP	30	8	12
256	Turbid	IP	21	6.5	12.5
72	Turbid	IP	21	5	17
255	Turbid	IP	31	6.5	18.5
180	Turbid	IP	33	8	25
429	Supernatant	IP	20	8	12
442	Supernatant	IP	27	11	12
443	Supernatant	IP	28	13	14
546	Supernatant plus flour	IP	37	19	20
599	Expressed	IP	30	17	18
155	Supernatant	SC	25	6	19

It is obvious from the size of some of these tumors (the largest weighed 65 gm.) that they must have been present for weeks or months before the animals were killed, and that the latent periods given in Table V are incorrect. However, there was no way of diagnosing with accuracy the presence of a tumor other than by autopsy, and the figures are intended only as rough approximations.

Some rats that never developed a tumor received as much as 42 cc. of oil intraperitoneally, and many received more than 30 cc.

Reference to Table IV will show that the final incidence of tumors was appreciably lower than the early figure of 25 per cent. On grouping the figures for turbid and "concentrated" oils, we obtain an incidence of 11 per cent among 91 rats that survived 9 months or more. The figure for supernatant oil and supernatant oil plus flour is 6 per cent of 67 rats. And if the rats that received Second Extraction oil are excluded, the figures are 6 per cent for subcutaneous, and 8 per cent for intraperitoneal injection of all types of oil.

DISCUSSION

In 1943, Rowntree and Ziegler (20) reported production of tumors in 11 of 46 rats that received repeated intraperitoneal injections of various types of wheat germ oil, and in 1 of 3 rats similarly injected with sesame oil containing 2 per cent starch and 2 per cent sterols derived from wheat germ oil. Three of these tumors were carcinomas, 1 was unclassified, and the other 7 were sarcomas.

Barry and Cook (2) in 1934 reported the development of tumor-like masses in 2 of 10 rats that had received repeated subcutaneous injections of lard. No tumors developed in similarly treated mice. Burrows, Hieger, and Kennaway (6) in 1936 reported the development of sarcomas in 7 of 102 rats repeatedly injected subcutaneously with lard, and in 1 of 28 rats treated similarly with olive oil containing a small amount of arsenious acid. Subcutaneous injection into mice yielded no tumors. Repeated intraperitoneal injection of lard or olive oil into 60 rats and 80 mice was followed by development of lymphosarcoma in 1 rat and 2 mice, but no spindle cell tumors appeared. Sarcomas developed in 2 of 14 rats injected with extracts of lard heated to 340-360° C. for 2 hours. In neither of these papers is there a statement as to the total amount of lard or oil administered.

At first thought it might appear that ether-extracted wheat germ oil administered by injection is a more potent carcinogen than lard. However, since we do not know the total volume of lard injected by the British investigators, we cannot say that this conclusion is warranted.

There are reports in the literature of the failure to produce tumors by single injections of small amounts of the fatty substances used as solvents for carcinogenic hydrocarbons, but we have not found any instances in which these substances have been injected as persistently as in our experiments and those of Rowntree and Ziegler (20). Possible exceptions are the reports of Barry and Cook (2), and of Burrows, Hieger, and Kennaway (6).

Not directly comparable to our experiments, but nevertheless of interest, are reports by Beck (3) on the production of sarcoma in 2 of 10 mice injected subcutaneously with 0.5 cc. doses of cottonseed oil that had been heated to 340-360° C. for 1 hour; and by Steiner, Steele, and Koch (23) who obtained sarcomas in 3 of 31 mice that received subcutaneously at 4-week intervals three 0.5 cc. doses of sesame oil that had been heated to 350° C., and in another experiment obtained a sarcoma in 1 of 20 mice injected subcutaneously with fat obtained by benzene extraction of overfried mixed meats. Five doses of 0.5 cc. were given at intervals of 4 weeks.

It is obvious that ether-extracted wheat germ oil is more carcinogenic than is oil obtained by expressing the seed, but we cannot assert that wheat germ is unique in this respect. Perhaps similar extraction of a large variety of seeds would produce oils that also would prove carcinogenic upon injection. In the light of present evidence, we think the best interpretation of the carcinogenic action of injected wheat germ oil is that the reaction is a non-specific one to chronic irritation. The production of tumors by overheated oils is presumably a result of the same mechanism.

SUMMARY

1. Despite prolonged oral administration of large amounts of ether-extracted wheat germ oil to rats, no tumors were obtained.

2. Repeated subcutaneous or intraperitoneal injections of the oil into mice and rats resulted in development of sarcomas in 2 mice and 16 rats. The tumor incidence was only 8 per cent in rats and 5 per cent in mice.

3. The production of these tumors is considered to be a non-specific reaction to chronic irritation.

ACKNOWLEDGMENT

I am indebted to Dr. G. H. A. Clowes for many helpful suggestions, and to Dr. L. G. Rowntree for his cordial co-operation in various aspects of this study.

REFERENCES

1. Auchincloss, R., and Haagensen, C. D. Failure of Wheat Germ Oil to Produce Neoplasms. Proc. Soc. Exper. Biol. & Med., **42**:703-705. 1939.

2. Barry, G., and Cook, J. W. A Comparison of the Action of Some Polycyclic Aromatic Hydrocarbons in Producing Tumours of Connective Tissue. Am. J. Cancer, **20**:58-69. 1934.

3. Beck, S. Sarcoma Produced by Subcutaneous Injections of Overheated Cotton-Seed Oil into Mice. Brit. J. Exper. Path., **22**:299-302. 1941.

4. Blumberg, H. Attempts to Produce Tumors in Rats by Feeding Crude Wheat Germ Oil Made by Prolonged Ether Extraction. Pub. Health Rep., **55**:531-537. 1940.

5. Brues, A. M., Marble, B. B., and Riegel, B. Failure to Induce Sarcoma in Rats with Wheat Germ Oil Preparations. Cancer Research, **1**:815-817. 1941.

6. Burrows, H., Hieger, I., and Kennaway, E. L. Experiments in Carcinogenesis: The Effect of the Subcutaneous and Intraperitoneal Injection of Lard, Olive Oil, and Other Fatty Materials in Rats and Mice. J. Path. & Bact., **43**:419-426. 1936.

7. Carruthers, C. Attempt to Produce Sarcomas in Rats from Ingestion of Crude Wheat Germ Oil by Ether Extraction. Proc. Soc. Exper. Biol. & Med., **40**:107-108. 1939.

8. Day, H. G., Becker, J. E., and McCollum, E. V. Effect of Ether Peroxides in Wheat Germ Oil on Production of Tumors in Rats. Proc. Soc. Exper. Biol. & Med., **40**:21-22. 1939.

9. Dingemanse, E., and Van Eck, W. F. Wheat Germ Oil and Tumor Formation. Proc. Soc. Exper. Biol. & Med., **41**:622-624. 1939.

10. Dorrance, G. M., and Ciccone, E. F. Production of Sarcoma in Albino Rats as a Result of Feeding Crude Wheat Germ Oil. Proc. Soc. Exper. Biol. & Med., **36**:426-427. 1937.

11. Evans, H. M., and Emerson, G. A. Failure to Produce Abdominal Neoplasms in Rats Receiving Wheat Germ Oil Extracted in Various Ways. Proc. Soc. Exper. Biol. & Med., **41**:318-320. 1939.

12. Ginzton, L. L., and Connor, C. L. Crude Wheat Germ Oil as a Factor of Tumor Formation in Rats. Am. J. Cancer, **38**:90-91. 1940.

13. Halter, C. R. Wheat Germ Oil and Tumor Formation. Proc. Soc. Exper. Biol. & Med., **40**:257-258. 1939.

14. Rider, D. L. An Experiment in Feeding Wheat Germ Oil Prepared by Ether Extraction. Am. J. Cancer, **38**:275-276. 1940.

15. Rowntree, L. G., Lansbury, J., and Steinberg, A. Neoplasms in Albino Rats Resulting from the Feeding of Crude Wheat Germ Oil Made by Ether-Extraction. Proc. Soc. Exper. Biol. & Med., **36**:424-426. 1937.

16. Rowntree, L. G., Steinberg, A., Dorrance, G. M., and Ciccone, E. F. Sarcoma in Rats Resulting from the Ingestion of a Crude Wheat Germ-Oil Made by Ether-Extraction. Tr. Assoc. Am. Physicians, **52**:344. 1937.

17. Rowntree, L. G., Steinberg, A., Dorrance, G. M., and Ciccone, E. F. Sarcoma in Rats from the

Ingestion of a Crude Wheat Germ Oil Made by Ether Extraction. Am. J. Cancer, **31**:359-372. 1937.

18. ROWNTREE, L. G., STEINBERG, A., DORRANCE, G. M., and CICCONE, E. F. Sarcoma in Rats Resulting from the Ingestion of Crude Wheat-Germ Oil Made by Ether Extraction. Pennsylvania M. J., **41**:784-788. 1938.
19. ROWNTREE, L. G., STEINBERG, A., and BROWN, W. R. The Production of Malignant Tumors in Rats with Wheat Germ Oil Made by Extraction with Ether. Tr. Assoc. Am. Physicians, **53**:199-201. 1938.
20. ROWNTREE, L. G., and ZIEGLER, W. M. Further Report on Malignancy Observed in Rats Injected with Crude Ether-Extracted Wheat Germ Oil. Proc. Soc. Exper. Biol. & Med., **54**:121-123. 1943.
21. RUSCH, H. P., BAUMANN, C. A., and MAISON, G. L. Production of Internal Tumors with Chemical Carcinogens. Arch. Path., **29**:8-19. 1940.
22. SANNIÉ, C., and TRUHAUT, R. L'ingestion d'huile de germe de blé peut-elle provoquer le cancer? Compt. rend. Soc. de biol., **131**:600-601. 1939.
23. STEINER, P. E., STEELE, R., and KOCH, F. C. The Possible Carcinogenicity of Overcooked Meats, Heated Cholesterol, Acrolein, and Heated Sesame Oil. Cancer Research, **3**:100-107. 1943.

Production of Sarcoma in Rats with Light Green SF

Paul N. Harris, M.D.

(From the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis 6, Indiana)

(Received for publication August 22, 1946)

In 1937 Schiller (1) reported that the dye, light green SF, was carcinogenic for rats but not for mice. In his experiment, 7 rats (white, and spotted black and white) received, once or twice weekly, subcutaneous injections of 1 to 3 cc. of an aqueous 2 per cent, and ultimately a 3 per cent, solution of the dye. When the animal's condition deteriorated, the injections were omitted or the volume was diminished. After about a year, 4 of the rats developed sarcomas at the site of injection.

As Schiller's experiment was conducted with a small number of heterogeneous animals, and since no confirmation had appeared in print, it seemed desirable to repeat Schiller's experiments, using a sample of light green SF supplied by Coleman and Bell.

METHODS

Our rats were obtained from a local breeder and were descended from the Wistar strain. Injections were made subcutaneously over the abdomen as nearly as possible in the same region every time. The first injection consisted of 2 cc. of a 2 per cent aqueous solution of light green SF, but this prostrated the animals, and the volume of subsequent doses was reduced to 1 cc. Injections were usually given twice weekly. After 1 month (9 treatments), the concentration of the dye solution was increased to 3 per cent, and following this increase the animals' condition at times became so poor that it was deemed advisable to omit a scheduled treatment.

As a result of the repeated injections, there was a gradual increase in subcutaneous fibrous tissue which ultimately made injection more difficult. Circumstances forced the discontinuation of injection after the 33rd week. During this time, each rat received 59 doses, or a total of 1.7 gm. of dye. In order to minimize leakage of the inoculum, injections were made with 24 or 25 gauge needles, but in spite of this, some leakage always occurred, so that none of the animals retained all of the amount administered.

RESULTS

Soon after completion of an injection the rats turned diffusely green, but as the dye was eliminated by the kidneys, their color returned to normal. However, the subcutaneous tissues about the site of injection soon became deeply stained, and some green color persisted for many months after cessation of treatment. The kidneys, too, became deeply pigmented. The dye was deposited in cells of the convoluted tubules and persisted for several months after treatment was stopped, but ultimately was eliminated.

The experiment was begun with 30 rats, but 4 died within 8 weeks, and 2 more died within 19 weeks after treatment was started. Fifteen rats developed tumors at the site of inoculation at the following intervals after the first injection: 35, 36, 37, 43, 44, 44, 48, 51, 56, 57, 59, 66, 83, 84, and 85 weeks. Nine rats died tumor-free 45, 57, 59, 61, 67, 71, 81, 85, and 92 weeks after the first injection. Hence, tumors developed in 63 per cent of the effective total of 24 rats.

Some tumors were easily separated from both skin and abdominal muscle; others were closely adherent to both structures, and still others were adherent to the skin only. Three of the last-mentioned were associated with slight ulceration of the skin. All tumors were fairly well circumscribed and rounded, and consisted of slightly translucent, whorled gray tissue. Most of the tumors grew rapidly after they became manifest. Microscopically, the tumors were fairly uniform and consisted of fusiform cells arranged in whorls and interlacing bundles. Mitoses were numerous in most tumors, and in only a few were giant cells at all common, although nearly all tumors contained some. Although some cells in a few of the tumors superficially resembled smooth muscle cells, no myofibrillae could be demonstrated, and all tumors were classed as fibrosarcoma. Typical examples of the tumors are shown in Figs. 1 and 2.

DISCUSSION

The question whether the tumors were produced by the dye or by some impurity is pertinent. Unfor-

FIG. 1.—Tumor from rat 16. The cells are spindle shaped, uniform, and loosely packed. Phosphotungstic acid hematoxylin. Mag. × 270.

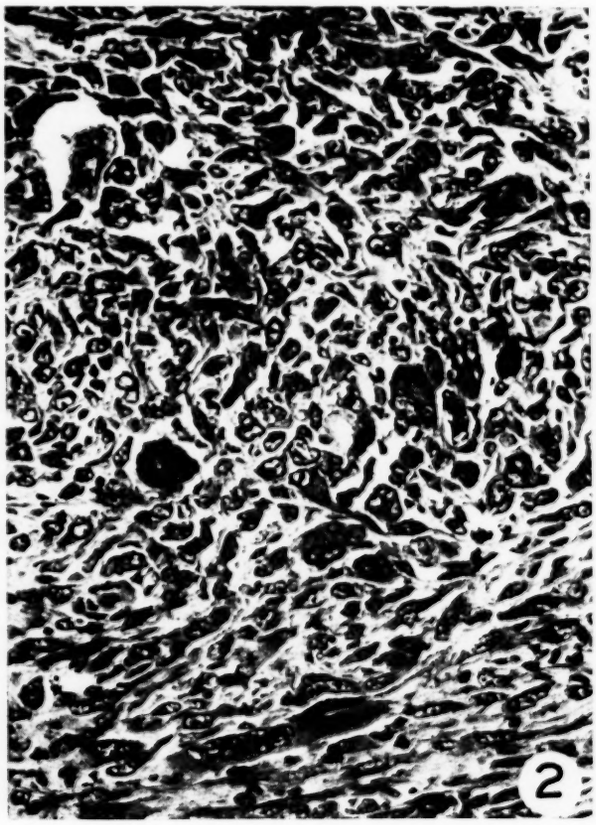
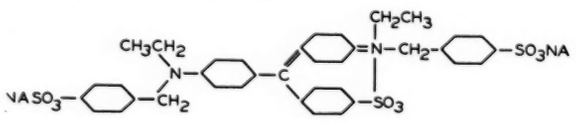


FIG. 2.—Tumor from rat 9. This tumor is more compact and the cells are much more variable. Phosphotungstic acid hematoxylin. Mag. × 270.

tunately, we cannot answer this question. An inquiry to Coleman and Bell as to the purity of the dye and the identity of impurities elicited the following reply, "We purchase this dye commercially and purify it for use as a biological stain. . . . The dye is a very complicated organic compound and we doubt if it could be prepared in a purity comparable with C.P. chemicals. It is reasonable to believe that it contains other organic compounds as impurities. What these compounds are and how much we are unable to say." The complexity of the structural formula of light green as given by Conn (2) is shown by Fig. 3.



LIGHT GREEN SF C.I. NO. 670

FIG. 3.

It seems probable that a higher incidence of tumors would have been obtained in our experiment if administration of the dye had been continued longer. However, the development of sarcomas in nearly two-thirds of the effective total number of rats treated indicates that, under proper conditions, the dye is a potent carcinogen.

SUMMARY

The reported carcinogenicity of a commercial sample of the dye, light green SF, administered by subcutaneous injection is confirmed. Sarcomas ultimately developed at the site of injection in 15 of 24 rats that received a total of 1.7 gm. of the dye in aqueous solution over a period of 33 weeks, but it is uncertain whether the carcinogenic action is attributable to the dye or to an impurity.

REFERENCES

1. SCHILLER, W. Rat Sarcoma Produced by the Injection of the Dye, Light Green F. S. Am. J. Cancer, **31**:486-490. 1937.
2. CONN, H. J. Biological Stains. Fourth edition. Geneva, N. Y.: 1940, p. 109.

A.A.A.S. Gibson Island Research Conference on Cancer, 1946

Abstracts of Papers Presented

On August 12 to 16, 1946, sessions of the summer meeting of the Section on Chemistry of the American Association for the Advancement of Science, at Gibson Island, Maryland, were devoted to a research conference on cancer. Dr. Harold P. Rusch, Professor of Oncology and Director of the Department of Cancer Research of the University of Wisconsin was Chairman, and Dr. Austin M. Brues, of the Argonne National Laboratory, University of Chicago was Vice-Chairman. Although there was no single topic of the conference many of the papers dealt with problems of carcinogenesis. Among the papers there were reports on the biological effects of radioactive compounds and nitrogen mustards. Abstracts of the papers presented follow.

STEROID HORMONES IN THE INDUCTION OF CANCER.* W. U. GARDNER. (Department of Anatomy, Yale University School of Medicine, New Haven 11, Connecticut.)

Estrogens are the only normally occurring steroid hormones, if not the only known chemically identified substances, produced within the body, the application of which results in cancer under specific conditions. Because nonsteroid, synthetic estrogens have carcinogenic effects similar to the normally occurring estrogens, the neoplasia-inciting action is associated with the biological responses rather than with an independent, specific chemical structure. Therefore the collective term, estrogen, is used in the following abstract although nine different estrogenic chemicals have been used in most of the experiments enumerated below.

In mice tumors of the pituitary gland, lymphoid tissue, testis, mammary gland and uterine cervix have appeared subsequent to estrogen-treatment, and in animals in which they would not have appeared unless given estrogens. With few exceptions, however, inherited factors or influences transmitted from parent or parents to offspring are of great significance. Estrogen-treated mice of one strain give rise to tumors of one or more tissues or glands but not of others. In this respect the estrogens are not unique; the carcinogenic hydrocarbons also produce different responses in mice with different genetic backgrounds and certainly differ greatly in effectiveness in different species. It is not improbable that two human beings differ more from one another than mice from one strain differ from mice of another strain. That a stimulus capable of eliciting neoplasia in one individual is inadequate in another is not improbable.

Pituitary tumors.—Almost all mice of the C57 strain, but very few mice of other strains, acquire chromophobe adenomas and/or extensive hypertrophies (12 to 260 mgm., whereas the mouse's pituitary gland normally weighs about 2 mgm.) of their pituitary glands when given estrogens for long periods of time. Although the males are more susceptible to the tumors than females both male and female mice of the C57 strain transmitted the tendency for pituitary tumors to their estrogen-treated hybrid young (C57 strain × CBA strain or C3H strain). Young obtained by backcrossing the hybrids to the parent stocks showed that the tendency for pituitary tumors among estrogen-treated mice is genetically transmitted, probably by multiple factors. Pituitary tumors have not appeared in the untreated mice of these strains.

Androgens inhibit but do not entirely prevent the development of pituitary tumors in estrogen-treated mice.

Several pituitary tumors have been transplanted. They have grown only in related and estrogen-treated hosts. The incidence of grafts that grow is low, growth is slow and delayed and no transplants could be carried for over 3 generations. The original tumors appear to continue growth after discontinuance of estrogen treatment because mice removed 138 days after cessation of injection still had large tumors; as large as those of mice continuously treated.

Lymphoid tumors.—Among 7 strains of mice with low incidences of lymphoid tumors in control animals, the estrogen-treated mice of 3 strains showed an augmented incidence of such tumors (15 per cent); of two strains a slight increase (5 per cent); and of two strains no effect. Large doses of estrogens were more lymphomogenic in the susceptible strains than small doses. The discontinuance of estrogen-treatment after 10 weeks did not reduce the incidence of lymphoid tumors although the tumors did not appear for several months after cessation of treatment. The leukemogenic effect of estrogens was inhibited by the simultaneous administration of testosterone.

The hybrid offspring of mice of 2 strains susceptible to estrogen-induced leukemia acquired a very high incidence of such tumors (40 to 45 per cent). The incidences of leukemia were intermediate in hybrid offspring of mice of strains susceptible to and not susceptible to estrogen-induced leukemia, and were equivocal when hybrids with parents of two non-responsive strains were studied. All lymphoid tumors were transplantable into untreated and related mice.

Carcinogenic hydrocarbons do not induce increased incidences of leukemia in mice of the strains used in which estrogens proved leukemogenic.

Testis tumors.—Tumors of the interstitial cells develop in the testes of estrogen-treated mice of the A and JK strains although such tumors occur rarely in untreated mice. The tumors metastasize to the lumbar and renal nodes, and occasionally to the lungs. They are transplantable into estrogen-treated hosts of the same strain for many transfer generations. In this laboratory they

* The investigations of the author referred to here were supported by grants from The Jane Coffin Childs Fund for Medical Research and The Anna Fuller Fund. Several of the experiments mentioned have been undertaken in collaboration with other investigators in the laboratory, namely, Dr. T. F. Dougherty, J. H. Dougherty, Dr. C. W. Hooker, Dr. C. A. Pfeiffer and Dr. L. C. Strong.

have not grown in untreated mice. Fragments of transplanted tumor tissue may remain dormant for several months in untreated hosts and start growth when estrogens are administered.

Both male and female mice of susceptible strains transmit the tendency for testicular tumors to their hybrid young. Three untreated hybrid male mice (A × C3H and A×C57) have also acquired testicular tumors at advanced ages.

Mammary tumors.—Estrogen-treated male mice from genetically susceptible strains and with the maternally transmitted mammary tumor inciter acquire mammary adenocarcinomas (Table I). The incidence is usually lower than among repeatedly bred females. Among the mice with genetic susceptibility but without the mammary tumor inciter the tumors appear at advanced age, grow slowly, and many are of the squamous cell type. Estrogen-treated male and female mice of the tumor-susceptible C3H strain acquired few mammary tumors when large doses of testosterone propionate were administered simultaneously. Not only was the incidence of mammary tumors reduced but localized overgrowths of the mammary glands seldom appeared and even mammary duct growth was subnormal.

TABLE I: INFLUENCE OF SEVERAL FACTORS IN MAMMARY CARCINOGENESIS

Strain of origin	Hybrid group	Average chromatin of susceptible strain, (%)	Mammary tumor inciter	Percentage mammary tumors: Estrogen-treated	Percentage mammary tumors: "Forced bred" controls
C57 ♀ × CBA ♂	CC_1	50	—	0	43
CBA ♀ × C57 ♂	CC_2	50	+	59	85
CC2 ♀ × CBA ♂	CC_3	75	+	54	79
CC1 ♀ × CBA ♂	CC_4	75	—	14	30
CC2 ♀ × C57 ♂	CC_5	25	+	1	7

In order to determine how the mammary tumor inciter might get into the milk and hence be transmitted to the offspring, studies were made on the milk which revealed formed elements including macrophages. Counts of cells in the blood obtained from the tail showed that during active lactation the white blood cells were almost 50 per cent below that of the non-nursing mouse (Table II). Lactating mice given large doses of estrogen had high white blood cell counts on the 4th day of treatment, at which time the weights of their nursing young began to decline.

TABLE II: THE AVERAGE TOTAL WHITE BLOOD CELL COUNTS OF LACTATING AND CONTROL MICE UNDER DIFFERENT CONDITIONS OF LACTATION

Group	No. of mice	Total number white blood cells: First count	24 hours	27-28 hours
1. Lactating control-suckling interrupted and not resumed	10	10,740(66)*	15,610(62)	16,185(62)
2. Lactating-suckling interrupted and resumed	18	7,480(65)	12,970(72)	6,850(66)
3. Control-non-lactating females or males	13	13,633(76)	12,597(71)	12,177(68)

* ( ) Percentage lymphocytes.

Uterine cervical tumors.—Carcinomas appear in the uterine cervices or upper vaginas of estrogen-treated mice of all strains that have been studied, but have not been observed among untreated mice except for one strain. The tumors usually appear, as judged by the detection of small infiltrative epithelial lesions, in the lower cervical canal, external cervix or vaginal fornix. Many stages of development, ranging from small tumors to large, and metastasizing cancers have also been observed. Several untreated mice of one stock, which shows a high incidence of imperforate vagina, have acquired cervical carcinoma; estrogen treatment augments the incidence in mice of this stock.

Simultaneous administration of testosterone propionate and estrogen did not prevent uterine cervical tumors. The tumors, however, showed less tendency to show cornified cells or pearls.

Uterine leiomyosarcomas have been noted in the uteri of several very old mice but not among estrogen-treated animals. Some overgrowth of uterine glands, with extension to the serosa (adenomyosis), is commonly observed but primarily malignant growths of the uterus proper have not been found.

By proper hybridization it is possible to combine transmitted tendencies so that any two or more types of tumors may appear in animals of the same group or even in the same animal within limitations of sex (Table III). Pituitary, lymphoid and mammary; or testicular, pituitary and mammary tumors have been found in single estrogen-treated hybrid mice. Other than for mammary tumors, there is no evidence of any tendency for tumors that is transmitted specifically by mice of one sex or the other

TABLE III: THE RELATIVE TENDENCY FOR ESTROGEN-TREATED MICE OF SEVERAL STRAINS TO ACQUIRE TUMORS

Strain	Mammary	Lymphoid	Testicular Interstitial Cell	Pituitary	Uterine Cervix
C3H	++++	+++	−	−	++
CBA	+++	+++	−	−	+++
PM	−	++	−	±	++
A	+++	−	+++	−	++
C57	−	−	−	+++	+
C12I	++	±	−	−	++
JK	−	±	++	−	?

Testosterone is antagonistic to tumorigenesis of the mammary, pituitary and lymphoid tissues but not to tumors of the uterine cervix, or probably, of the testicular interstitial cells. No evidence of changes in the adrenal glands have been noted that could be associated with tumors of organs other than the pituitary gland.

TUMORS IN INTRASPLENIC OVARIAN TRANSPLANTS IN CASTRATED MICE.

M. H. LI and W. U. GARDNER. (Department of Anatomy, Yale University School of Medicine, New Haven 11, Connecticut.)

It has been shown that many steroid gonadal hormones are inactivated when circulated through the hepatic portal system, and that the intrinsic production of gonadotrophins is increased subsequent to castration. This investigation demonstrated the role of the endogenous gonadotrophic hormones of the pituitary gland in ovarian tumor formation by the transplantation of ovary into spleens of castrated mice.

Inbred mice of the Strong A and C3H strains, and

hybrid mice (AC3, AC5) were used. Six groups of experiments were set up: (a) Twenty-one castrated male mice with homotransplantation of an ovary into the spleen; (b) 52 castrated female mice with autotransplantation of an ovary into the spleen; (c) 25 castrated female mice with subcutaneous autotransplantation of an ovary into the right axillary region; (d) 12 intact male mice with subcutaneous homotransplantation of an ovary into the right axillary region, (e) castrated male mice with subcutaneous homotransplantation of an ovary either into the right axillary region or into the abdominal region; and (f) 33 intact male mice with homotransplantation or heterotransplantation of an ovary into the right testis. Castration and grafting were done when the mice were 1 to 3 months old, except in the group (f) in which a few mice were operated upon at 130 days of age. The donors of the ovaries for homotransplantation or heterotransplantations were usually younger than the recipients.

Five granulosa-cell tumors, 2 probably tumors of the same type and 1 mixed tumor consisting of both granulosa and luteomatous cells were found among the 21 castrated male mice with intrasplenic ovarian transplants. Four luteomas and 7 mixed tumors were found among the 33 castrated female mice that showed no adhesion of the intrasplenic ovarian graft to the left uterine horn or to the adjacent peritoneum. Among the 19 castrated female mice with vascularized adhesions of the ovarian transplant 1 luteoma was observed in a mouse that had irregular estrous cycles during the latter part of the experimental period. Mice of the A strain were used in these 2 groups of experiments and most of the ovarian tumors developed in castrated mice carrying the intrasplenic ovarian transplants for more than 7 months. No ovarian tumors were found in other sites of transplantation except for one small granulosa cell tumor-like growth observed in a subcutaneously transplanted ovary in a castrated female mouse. The mouse was the only one that was operated upon during pregnancy.

THE METABOLISM AND CARCINOGENICITY OF *p*-DIMETHYLAMINOAZOBENZENE AND RELATED COMPOUNDS IN THE RAT.

J. A. MILLER and E. C. MILLER. (McArdle Memorial Laboratory, Medical School, University of Wisconsin, Madison 6, Wisconsin.)

These studies cover some of our efforts to define the carcinogen operative in carcinogenesis by the aminoazo dyes through investigations on their metabolism and the effect of structure on their activity.

Using a sensitive quantitative method for the determination of basic aminoazo dyes in tissue we have demonstrated that one of the initial pathways in the metabolism of *p*-dimethylaminoazobenzene (DAB) is its reversible demethylation to *p*-monomethylaminoazobenzene (MAB) followed by an irreversible demethylation to *p*-aminoazobenzene (AB) (3, 4). These basic aminoazo dyes were initially characterized by their absorption spectra and adsorption characteristics. We have further characterized the dyes by reduction to their component amines which were then determined by an independent method described below. DAB and MAB have been found to be metabolically and carcinogenically interchangeable (6). In animals fed these compounds the liver is the only organ that contains appreciable amounts of all 3 dyes. The tumors arising in livers containing the basic dyes contain similar amounts of these compounds. AB is temporarily stored in the red blood cells and is also excreted in the bile. The same amount of AB appears in the blood after any of the 3 dyes are fed; this seems to indicate that the metabolism of DAB and MAB proceeds largely through an initial demethylation to AB. The amount of AB in the blood varies directly with the concentration of dye in the diet. The dye levels in the tissues reach a maximum in about a week of feeding. When the dye is withheld from the diet the dye contents of the tissues drop about 50 to 90 per cent in 3 days and in a week very little is left. The basic aminoazo dyes found in the body at any one time could account for about 8 per cent of the dye ingested within the previous day. None of the various diets known either to increase or decrease the rate of hepatic tumor formation with DAB have been found to alter greatly the levels of dye in the liver and blood.

A study of the over-all metabolism of DAB, particularly the dyes and their constituent amines that may be found in the urine has also been made. In general our observations on the over-all metabolism of DAB agree with those of Stevenson and her group (7) in that *p*-phenylene diamine and *p*-aminophenol appear to be quantitatively the major final metabolites of the dye. The application of a sensitive and relatively specific method for the determination of six monophenyl amines in urine has disclosed the presence of minor quantities of other metabolites. In addition a new class of aminoazo dyes appears to be present. The urine of a rat fed DAB contains much less basic aminoazo dye than can account for the red color given with acid. The urine becomes orange when made alkaline but the color cannot be extracted with petroleum ether or benzene. These properties indicated that one or more amphoteric aminoazo dyes were present. No hydrolysis to simpler azo dyes has proven possible and a means has not been found for their removal from urine uncontaminated with monophenylamine. Instead, we have had to rely on an approximate means for their estimation. This consists in reducing the azo dyes in the urine in the cold at an acid pH with $Na_2S_2O_4$ and then determining the free amines formed. This procedure determines only free amphoteric dyes and the free portions of conjugated dyes. It was necessary to release the conjugated monophenyl amines present in the urine for analysis by hot acid hydrolysis under reducing conditions. The 3 basic aminoazo dyes and their *p'*-hydroxy derivatives, which are representative amphoteric aminoazo dyes, were found to be reduced quantitatively to their constituent amines by refluxing in dilute HCl in the presence of tin. Hence the monophenyl amines were determined by the difference between the total amines found after reductive hydrolysis in acid and the amines found after reduction in the cold.

Four aromatic amines could arise in the reduction of the basic aminoazo dyes, *viz.*, dimethyl-*p*-phenylene diamine (DMPD), monomethyl-*p*-phenylene diamine (MMPD), *p*-phenylene diamine (PD), and aniline (A). *p*-Aminophenol (*p*-AP) was already known as a metabolite of DAB. After a method had been devised for the estimation of these amines, it became evident that still another aminophenol, *o*-aminophenol (*o*-AP), was present in the urine. The method employed allows the separation and estimation of these 6 amines in microgram quantities as colored complexes through an application of the Böniger reaction. Primary aromatic amines were found to react instantly in the cold at pH 7 with excess sodium-β-naphthoquinone-4-sulfonate to form highly col-

ored phenolic Schiff bases. Benzene will extract the Schiff bases formed with DMPD, MMPD, *o*-AP, and A. The first two amines form blue and purple solutions respectively and the MMPD base may be selectively destroyed with acetic anhydride. *o*-AP and A form orange and yellow solutions respectively. Hence these four Schiff bases in the benzene extract can be separately determined by measurements of light absorption at different wave lengths before and after the addition of acetic anhydride. PD forms a highly insoluble Schiff base which can be quantitatively filtered on asbestos and then dissolved therefrom by dilute base to form an orange-red solution. The extracted and filtered aqueous solution still contains the *p*-AP base, which may then be extracted with amyl acetate in which it forms an orange solution. The interference by normal urine constituents is low and satisfactory recoveries of added amines were obtained.

Each of the 3 basic aminoazo dyes, the three *p'*-hydroxy aminoazo dyes, and the six monophenyl amines have been fed to rats and the urines examined for these compounds and their derivatives. When free amines were excreted the urines were collected through funnels coated with hydroquinone and caught in dilute HCl in the presence of either tin or hydroquinone and benzene. Approximately 50 per cent of each of the azo dyes could be accounted for in the urine in terms of the azo dyes and amines found. While the major metabolites in each case were PD and *p*-AP, small amounts of MMPD, A, and *o*-AP were noted. Very small quantities of DMPD, close to the limit of the method, appeared to be present in the urines of rats fed the dimethyl- and monomethylaminoazo dyes but were not noted in the urines of rats fed either of the completely demethylated dyes. Basic azo dyes were found only in the urines of rats fed the basic dyes. Amphoteric azo dyes were found in the urines of rats fed any of the 6 azo dyes. The probable composition of the amphoteric azo dyes in the urine is indicated by the observation that from the urines of rats fed DAB and MAB the amines MMPD, PD, *p*-AP, and *o*-AP were formed after cold acid reduction. The urine of rats fed AB yielded PD, *p*-AP, and *o*-AP under such conditions. After reduction, the urine of rats fed the *p'*-hydroxy derivatives contained similar compounds except that no *o*-AP was noted. The amphoteric aminoazo dyes appear to account for only a few per cent of the azo dye fed. No free monophenyl amines were found in the fresh urines of rats fed any of the 6 azo dyes. When the urine of azo dye-fed rats was allowed to stand at room temperature without a preservative the amphoteric aminoazo dyes disappeared and free PD and *p*-AP appeared. A different situation was found when the monophenyl amines were fed since considerable free amine was excreted in each case although the majority was in the conjugated form. Seventy-three or more per cent of each of the diamines was recovered in the urine. Although most of the DMPD and MMPD fed was demethylated to PD appreciable amounts of DMPD and MMPD were found in the urine after feeding DMPD. MMPD yielded only MMPD and PD and only PD was found in the urines of rats fed PD. When A was fed it was metabolized to *p*-AP although some *o*-AP and A were excreted. Each aminophenol when fed yielded only that aminophenol in the urine.

The above data indicate that a considerable portion of the metabolism of DAB probably proceeds through the basic aminoazo dyes to the amphoteric aminoazo dyes and thence to the monophenyl amines. A minor pathway from AB direct to A and PD probably exists also. There is as yet no evidence for deamination at any step, rearrangement of hydroazo compounds, or for the direct reduction of the methylated basic aminoazo dyes since the MMPD and the small amount of apparent DMPD in the urine could also come from the reduction of hydroxy aminoazo dyes. Preliminary data indicate that the over-all metabolism of DAB is not greatly altered by diets which significantly alter its carcinogenicity.

Our studies on the effect of structure on the carcinogenicity of the aminoazo dyes have been designed to define more clearly the structural features necessary for *strong* carcinogenic activity and to test the activity of possible metabolites of DAB. Each compound was fed to at least 10 rats at a molar level equivalent to 0.06 per cent of DAB, the reference compound, for 4 to 8 months in a diet which allows a high incidence of tumors with DAB. The compounds were rated on a rough scale of activity in which DAB is assigned a value of 6. Approximately 40 azo dyes and amines were tested and only a few of the azo dyes were found to possess strong activity. Alterations in the azo linkage, the ring substituents, and the substituents of the amino groups have been tested. When the azo linkage in DAB was replaced by $-N=CH-$, $-CH=N-$, or by $-CO-NH-$ (benzamide of DMPD) the activity was abolished. When the methyl groups in DAB were replaced by 2 H—, H— and CH_3CH_2—, or 2 CH_3CH_2— compounds of zero activity resulted. On the other hand if the amino group carried H— and CH_3— or CH_3— and CH_3CH_2— the activities were equal to that of DAB. In contrast to the latter case the azo dye with CH_3— and $HOCH_2CH_2$— on the amino group was inactive. Inactivity was also produced when the amino group substituents were CH_3— and phenyl—CH_2— or H— and CHO—. Ring substituents produced great variations in activity. When the ring hydrogens were substituted one at a time with methyl groups the activities of the compounds ranged from 0 to 10-12 (1, 5), in contrast to the prediction of Kensler and his co-workers (2). Although $-NO_2$ groups in the *o'*— and *m'*— positions reduced the activity of DAB, the *m'*—NO_2 compound, like the *m'*—CH_3 dye, was more active than the corresponding *o'*—derivative. None of the possible metabolites of DAB other than MAB have shown any activity. The *p'-hydroxy* derivatives of DAB, MAB, and AB were inactive. DMPD and A fed together were inactive even at 3 × the equivalent to 0.06 per cent DAB. MMPD, PD, A, *p*-AP, and *o*-AP were likewise inactive. 2,4'-Diamino-5-dimethylaminodiphenyl, 3-dimethylaminocarbazole, and 4-hydroxyazobenzene also proved to be inactive.

The conclusion drawn from the above studies is that the real carcinogen operative in carcinogenesis by DAB is either one or both of the two dyes, DAB and MAB, or some azo dye which is very close in its structure to these compounds.

Recently, we have found a tight combination, presumably chemical, between an unidentified aminoazo dye and a cellular constituent, probably protein, in the livers of rats fed DAB. The dye could not be extracted from isolated crude liver protein by boiling organic solvents, but it could be extracted with ethyl ether-ethanol after the protein had been degraded with either hot alkali or trypsin. For an approximate estimation of the "bound dye," tissue protein was digested with boiling alcoholic KOH and the digest was extracted with ethyl ether; after removing the solvent the residue was dissolved in alco-

holic HCl and the color intensity was read at 520 mμ. In rats fed DAB the bound dye was found only in the liver and not in the blood, lungs, spleen, heart, skeletal muscle, intestine, or kidneys. The livers of rabbits, guinea pigs, and cotton rats did not contain the bound dye while mouse livers had only about a fourth as much as rat livers. The livers of rats fed DAB and MAB appeared to contain similar quantities of the same bound dye(s) while the livers of rats fed AB had very little of this dye. These findings correlate well with published data on the specificity of DAB in producing tumors of the liver, on the inability of other workers to induce tumors in rabbits or guinea pigs, the lesser susceptibility of mice as compared with rats, and the extremely low carcinogenicity of AB. About a month was required for the level of bound dye to reach a maximum after feeding with DAB was initiated, but the bound dye content dropped approximately to zero within a few days after the rats were red dye-free diets. The tumors arising in livers containing the bound dye contained none of this compound. The data suggest that the bound dye may have an important place in carcinogenesis by the aminoazo dyes.

REFERENCES

1. Giese, J. E., Miller, J. A., and Baumann, C. A. The Carcinogenicity of m^1-Methyl-*p*-Dimethylaminoazobenzene and of *p*-Monomethylaminoazobenzene. Cancer Research, **5**:337-340. 1945.
2. Kensler, C. J., Dexter, S. O., and Rhoads, C. P. The Inhibition of a Diphosphopyridine Nucleotide System by Split Products of Dimethylaminoazobenzene. Cancer Research, **2**:1-10. 1942.
3. Miller, J. A., and Baumann, C. A. The Determination of *p*-Dimethylaminoazobenzene, *p*-Monomethylaminoazobenzene, and *p*-Aminoazobenzene in Tissue. Cancer Research, **5**:157-161. 1945.
4. Miller, J. A., Miller, E. C., and Baumann, C. A. On the Methylation and Demethylation of Certain Carcinogenic Azo Dyes in the Rat. Cancer Research, **5**:162-168. 1945.
5. Miller, J. A., and Baumann, C. A. The Carcinogenicity of Certain Azo Dyes Related to *p*-Dimethylaminoazobenzene. Cancer Research, **5**:227-234. 1945.
6. Miller, E. C., and Baumann, C. A. The Carcinogenicity of *p*-Monomethylaminoazobenzene in Various Diets and the Activity of this Dye Relative to *p*-Dimethylaminoazobenzene. Cancer Research, **6**:289-295. 1946.
7. Stevenson, E. S., Dobriner, K., and Rhoades, C. P. The Metabolism of Dimethylaminoazobenzene (Butter Yellow) in Rats. Cancer Research, **2**:160-167. 1942.

NUCLEAR DIFFERENTIATION AND THE ORIGIN OF TUMORS.

JACK SCHULTZ. (The Institute for Cancer Research, The Lankenau Hospital, Philadelphia 30, Pennsylvania.)

This is a review in which the basis for a concept of the differentiation of cell nuclei during development is discussed. It is shown that a new theory of the origin of tumors, based on this concept, can be developed without any additional *ad hoc* assumptions.

Examples of the correlative differentiation of the nucleus and cytoplasm are given under four categories. (a) Losses of parts of chromosomes during early development (*Ascaris*). (b) Increase in chromosome number without nuclear division: "endomitosis." (c) Differential reproduction of genes, during endomitosis. (d) Changes in state of chromosomes; increase in nucleoprotein content in pycnotic nuclei, relatively high protein content of "diffuse" nuclei. This last category of nuclear differentiation involves visible changes in the chromosomes, hence in the immediate vicinity of the genes, and possibly within the genes themselves. The differences between the same chromosome region in the two types of nuclei discussed are analogous to those found between the heterochromatic regions (rich in desoxyribosenucleic acid) and the euchromatic regions (relatively poor in this substance) within a single nucleus. The analysis of the genetic function of the two types of chromosome region provides a basis for a working hypothesis regarding nuclear differentiation. In particular, the combination of cytochemical and genetical studies in *Drosophila* gives evidence for the connection of the heterochromatic regions with the overall nucleoprotein metabolism of the cell: ribosenucleoprotein in the cytoplasm, desoxyribose in the chromosome. The euchromatic regions seem by and large, in this at present obviously oversimplified picture, to be concerned with the diversified syntheses that are diagnostic of different genes. In *Drosophila* there are many cases in which euchromatic regions placed next to heterochromatin change their properties; individuals carrying such chromosome rearrangements show somatic "mutation" for the genes in the abnormally placed euchromatin. These changes are effectively losses of the specific activities of the genes, and are correlated cytologically with the assumption of the properties of heterochromatin by the regions of chromosome involved, in the differentiation of the nuclei of the different tissues. There is thus a basis for the postulate that the changes in "pycnosis" of the different types of nuclei are reflections of changes in gene activity, and in extreme cases, of the genes themselves.

The extension of this postulate leads to the point of view that during development a process of genic "adaptation" takes place. This "adaptation" occurs concurrent with the establishment of a semi-autonomous system of elements in the cytoplasm, associated with cytoplasmic ribose-nucleoproteins. The genes whose specific activities are essential for the maintenance of the cytoplasmic system differ for the different cell types, and probably relatively few remain in the "active" (euchromatic) state in any one nucleus. The much larger remaining number of genes is adapted to a state of activity comparable to that of the heterochromatic regions, a state of generalized function concerned with gene reproduction and the mitotic process. The further the process of nuclear differentiation has proceeded, the more these adaptations are irreversible; and in the germ cell all genes are in the "adaptable" state almost by definition. It should be stated that this discussion is intended especially for the last of the four categories of nuclear differentiation; for the other types, where differentiation is obvious, its consequences for the discussion to follow are the same in principle.

On the assumption that an irreversible adaptation of genes for generalized and for specific function occurs during development, the nuclei of differentiated cells have lost an adaptability present in their embryonic forbears. Of the total number of genes present, the majority are conceived to be adapted for the generalized function; but since in the ordinary life of a differentiated cell, the stimuli for mitosis are either rare or highly specific and mediated by an already functioning cytoplasmic system.

the reactions in which they participate are conceived rarely to occur. Should, however, the established cytoplasmic system be broken down, and the cell not die, a new system must be regenerated. This regeneration of the cytoplasmic system takes place under the influence of the differentiated nucleus, with the preponderance of genes adapted for generalized function ("heterochromatin-adapted"). Such genes are maximally functional in embryonic tissue; consequently the type of cytoplasmic system regenerated will resemble that of the embryonic cell. Mitosis will occur; but since the nucleus is a differentiated one, without the "adaptable" genes of an embryonic cell, the daughter cells, instead of being responsive to "organizing" influences about them, will continue to produce the embryonic type of cytoplasm. Thus a cell type, committed under most circumstances to mitosis, and relatively unresponsive to the needs of differentiation about it, will arise. Its characteristics are those of the malignant cell.

Comparison of this hypothetical picture with the actual data strengthens a belief in its validity. (a) The multiplicity of stimuli evocative of tumors calls for an effect on some constituent of the cell. Recent cytological work (see especially Opie) shows a stage in tumor development to be the breakdown of the nucleoprotein granules of the cytoplasm. Viruses, on this view, act by the cytoplasmic damage they cause, and propagate in the tumor cell at the expense of its embryonic cytoplasm. The precancerous lesions have the characteristics of embryonic tissue. (b) The nuclei of tumor cells resemble most the more differentiated of the nuclei in the tissue of origin (Biesele); their high nucleoprotein content (Stowell) indicates possible direct evidence for heterochromatic adaptation. (c) The direct chemical analyses of the constitution, the enzymatic activity, and the metabolism of tumor cells accord more closely with those of embryonic cells than with any other group. It seems likely, and in some of these cases it is already clear, that these measurements reflect similarities in the cytoplasms. (d) Other groups of data (antigenic properties, behavior on transplantation, relation of malignancy to tissue type of origin, differentiation in the tumor itself), insofar as available, are consistent with the view. Obviously many kinds of experiment are necessary, to test the basic assumptions, and to make precise the properties of the tumor cell in usable terms. The working hypothesis is tenable, that the malignant cell is the result of the combination of an "old" nucleus with "young" cytoplasm.

CARCINOGENESIS AND THE MECHANISM OF GENE ACTION.

S. SPIEGELMAN. (Department of Bacteriology, Washington University School of Medicine, St. Louis 10, Missouri.)

The basic problem of carcinogenesis involves explaining the appearance of a sudden *heritable* change in somatic cells which implies the involvement of the hereditary mechanism at some stage in the process. On this assumption it becomes imperative to examine the question from the point of view of what is known about the mechanism of gene action. Here one must emphasize the distinction between two quite different aspects of the gene problem. One involves simply their transmission from one generation to the next and the other the mechanism whereby genes effect their control over cellular physiology. It is with the latter problem that the present paper is primarily concerned.

Recent work employing micro-organisms as genetic materials has led to some rather well defined concepts on the nature of gene action. The basic assumption underlying much of this work is that since the enzymes of a cell determine its physiological capabilities, genes must control the phenotype of a cell by virtue of their ability to control enzymatic constitution. Adaptive enzyme formation and its inheritance in yeast was studied in an attempt to learn more about the nature and extent of this control.

We may summarize the important findings and conclusions relating to genes and enzyme formation by the following statements.

1. Usually, the transmission of characteristic enzymes and the products of their activities follows the classical Mendelian laws derived from the assumption that the controlling units are self-duplicating entities, genes, located on chromosomes in the nucleus.
2. The existence of a particular gene in the nucleus of a cell does not guarantee that the corresponding enzyme will be found in the cytoplasm, as evidenced by such phenomena as cellular differentiation and enzymatic adaptation. Genes, therefore, have as their primary function the indefinite retention for the cell of the potentiality for enzyme formation.
3. The actual formation of an enzyme in the cytoplasm is mediated directly by a cytoplasmic unit (plasmagene) which possesses the capacity for self-duplication in the presence or absence of the corresponding gene.
4. The presence of the homologous substrates accentuates the capacity of these self-duplicating plasmagenes to produce enzyme.
5. Competitive interactions exist amongst the cytoplasmic enzyme-forming units.
6. Nucleoproteins are involved in the synthesis of enzymes.

These findings and conclusions have been synthesized into a theory of gene action which assumes that genes *continually* produce at various rates more or less complete replicas of themselves which enter the cytoplasm. These replicas or plasmagenes are presumed to be nucleoprotein in nature in view of their origin and to possess to varying degrees the capacity of self-duplication. Their presence in the cytoplasm controls the types and amounts of proteins and enzymes synthesized. These plasmagenes, like all self-duplicating entities, would compete with each other for protein and energy, and the outcome of such competitive interactions would then determine the enzymatic constitution of the cytoplasm. Inherent in this concept is the possibility of changing the ultimate result of this competition by varying the conditions (*e.g.*, substrates available) under which it takes place. The various reactions and the rôle of substrate in the process are detailed in Fig. 1 in terms of one gene G_1 and its corresponding enzyme E_1.

All the double arrows denote self-duplicating reactions. Gene G_1 continually produces its plasmagenes (Pl_1) at a rate denoted by k. The plasmagene by its very nature must possess heterocatalytic potentialities, *i.e.*, in addition to being autosynthetic it must possess the capacity of catalyzing the synthesis of units (enzymes) other than itself. Consequently, once in the cytoplasm, several things may happen to plasmagene (Pl_1). If it is successful in obtaining the proper material (M) in the cytoplasm it will duplicate itself. It may, on the other hand, combine with precursor protein (Pr) and convert it to E_1, resulting in the formation of the Pl_1E_1 complex.

Since little enzyme is found experimentally in the absence of substrate, one must assume that this complex is

highly unstable and quickly breaks up into its two components. A plasmagene once formed cannot of course exist indefinitely, particularly in a population of other such units actively competing for the material of which it is composed. The reaction leading from Pl_1 to IP (inactive protein) in Fig. 1 describes this fact. Enzyme may

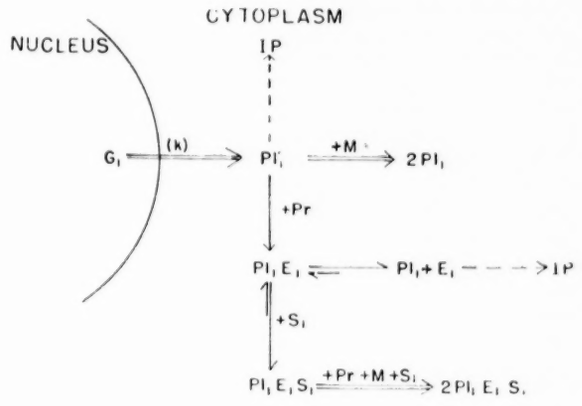


FIG. 1.—Diagram of theory of gene and plasma gene action. G_1=gene; Pl_1=plasmagene; Pr=enzyme precursor; E_1=enzyme; S_1=substrate; and M=cytoplasmic material used in duplication of plasmagene. The constant (k) is a reaction velocity constant measuring rate of production of plasmagenes from genes. All double arrows indicate a self-duplicating process; broken-line arrows denote decay to inactive protein (IP) of the element from which the arrow points. Where forward and backward reactions are denoted, the longer of the two arrows indicate the major tendency of the reaction.

also break down to inactive protein as is indicated by loss of activity or removal of substrate. By IP or inactive protein we mean merely that the plasmagene has broken down to a protein unit which has lost the capacities for self-duplication and enzyme formation, and in the case of enzyme, into a protein which no longer possesses enzyme activity.

Thus far we have described the reactions which take place in the absence of substrate. It is clear that little enzyme would be found in the cell, unless the rate of Pl_1 production by G_1 were extremely high or the stability of the enzymes or enzyme-plasmagene complex very great. Neither condition is apparently satisfied in the cases of the enzymes reported on here. When substrate S_1 is added, however, it will combine with E_1 and two things may result. It is well known that the addition of substrate to enzyme stabilizes it against inactivation. Hence the presence of substrate would decrease the rate at which free E_1 is converted to inactive protein. More critical however is the possibility that S would combine with E_1 while the latter is still united with Pl_1, thus resulting in a $Pl_1E_1S_1$ complex. The substrate would now not only stabilize the enzyme, but could also stabilize the unstable plasmagene-enzyme (Pl_1E_1) combination. Such stabilizations of unstable complexes by the addition of a third component are quite common in organic chemistry.

Inherent in the very definition of a self-duplicating entity is the concept that should such a unit undergo a modification at any given moment, all subsequent replicas would bear this modification. Thus when Pl_1 exists alone it *duplicates* only Pl_1. However, when substrate is added and the stable $Pl_1E_1S_1$ combination results *this* is now duplicated, which is indicated in the diagram by the double arrow. This scheme provides therefore a concrete mechanism whereby substrate can modify competitive interactions between plasmagenes. What is essentially accomplished by substrate is the creation of a new self-duplicating unit which duplicates not only the plasmagene but also the enzyme corresponding to the substrate added.

From a general point of view, the unique feature of the theory outlined above of gene action is that while supplying a link between the gene and the enzyme, it at the same time predicts that cells with identical genomes need not possess identical enzymatic constitutions. Whether a particular enzyme will be transmitted from one cell generation to the next in a Mendelian fashion will thus depend on the relative rates of duplication of the controlling cytoplasmic units as compared with their rate of production from the genome. If the latter is quantitatively determining, Mendelian inheritance will be observed. If the former is determining, the Mendelian picture will be obscured to varying degrees, depending upon the self-duplicating capacity of the plasmagenes. It is clear also how substrate could so intensify cytoplasmic inheritance of a particular enzyme as to completely obscure the segregation of the corresponding gene.

As a tentative working hypothesis this theory has the advantage of furnishing a unified point of view from which such diverse and apparently contradictory phenomena as classical Mendelian genetics, cytoplasmic inheritance, cellular differentiation, and enzymatic adaptation may be analyzed. Its implications for the cancer problem are evident. From the viewpoint of the plasmagene mechanism of gene action, heritable changes such as carcinogenesis need not necessarily be referred back to the genes. The plasmagene by being self-duplicating provides us with another level at which a mutation can take place and be subsequently transmitted via the cytoplasm from one cell generation to the next. This concept may also furnish a basis for reconciling the somatic mutation versus the virus theories of cancer, since, if the mutation does occur at the plasmagene level, the difference between these two opposing views becomes tenuous indeed.

PATHS OF GENE ACTION IN MAMMARY TUMOR DEVELOPMENT IN MICE.

W. E. HESTON. (National Cancer Research Institute, National Institute of Health, United States Public Health Service, Bethesda 14, Maryland.)

Now that genetic differences in the development of mammary tumors in mice have been demonstrated, the present problem is that of determining the physiologic mechanisms through which these variations in genic complex become manifest. From recent work on gene chemistry such genic differences can be expected to affect enzymatic differences controlling specific metabolic reactions related to the subsequent development of the tumor.

At present three paths through which differences in genic action affect the probability that a mammary tumor will appear have been identified. There is evidence suggesting that genic differences can influence the response of the mammary tissue cell to the hormonal stimulation and to the milk agent stimulation. In either case the gene action must occur in the mammary tissue cell. There is evidence that genic differences can influence the development of mammary tumors through the hormonal mechanism, possibly in controlling the production of the hormonal stimulation. In this path the gene action can be expected to be in the physiology of the endocrine system. Genic differences can influence the propagation and

transmission of the milk agent. Since it has not been demonstrated that the milk agent is limited to any particular tissue, one cannot predict just where the gene action occurs in this case. Through the effect on the transmission of the agent, gene action in one female may influence the probability that a tumor will appear in her daughter or in a foster-nursed female.

Possible bearings that these findings in the mouse may have on the investigations of breast cancer in the human being were discussed.

THE INDUCTION OF GERMINAL MUTATIONS BY A CARCINOGENIC CHEMICAL (METHYLCHOLANTHRENE). LEONELL C. STRONG. (Department of Anatomy, Yale University School of Medicine, New Haven 11, Connecticut.)

Fifty-two germinal mutations affecting intensity and distribution of pigment and spotting patterns of the hair of mice have been obtained in the descendants of mice, whose ancestry has been injected with methylcholanthrene over a number of generations. None of these mutants have occurred in mice of the untreated control series. In the series treated earlier (4 generations), no germinal mutations were obtained and the mice, as a rule, died early following the injection of methylcholanthrene, with the progressive growth of local tumors appearing at the site of injection. However, selection toward resistance to tumors induced by methylcholanthrene has resulted in the suppression of local tumors, as well as other types of internal tumors whose latent periods are considerably longer than the ones obtained with local tumors at the site of injection. When the effect of selection toward resistance to tumors had been great enough to permit the appearance of abdominal tumors (with long latent periods) such as primary carcinoma of the liver, adenocarcinoma of the gastric mucosa, leiomyosarcoma of the uterus, and granulosa cell carcinoma of the ovary, the descendants of these selected mice began to show many germinal mutations.

At present, it is intended to discuss only three groups of these mutants. (A) There has apparently appeared a series of alleles at the brown-black locus. Not all genetic tests have been made on all these mutants but apparently each type breeds true and segregates following an outcross. Any mouse may have one or two of these genes but no more. A cross between two of these new mutants gives one or the other of the new mutants, not the wild type. This series of alleles (new mutations) classified according to intensity of pigmentation by visual inspection is (1) black (established dominant = BB), (2) graphite, (3) dark gunmetal, (4) gunmetal, (5) dark bronze, (6) bronze, (7) darker sepia, (8) dark sepia, (9) sepia, (10) chestnut, (11) brown (established recessive = bb), (12) light brown, (13) lighter brown, (14) velvet, and (15) buff. (B) Another series of mutations involving pigmentation produces a lighter ventral area than occurs on the dorsum. This series has occurred in mice whose ancestry had shown nothing for many generations but non-agouti, where there is no differential pigmentation on the dorsum and ventrum. Thus there has been obtained a series of mutants classified according to pigmentation on the ventrum of (1) black or non-agouti, (2) light black, (3) rusty, (4) red, (5) chestnut, (6) tan, (7) sandy, (8) derris and (9) buff. (C) The third series of new mutants involves the formation of different colored spots on either the forehead or ventrum. The colors of spots have been, (1) white, (2) gray, and (3) yellow.

In addition to these clear-cut germinal mutations there has been obtained many somatic mosaics between any two of the above color mutants; such as between buff and brown, chestnut and brown and rusty and graphite, etc. Many of the new color mutations are permanent from the growth of the first hair until old age, while others shift more and more toward the recessive condition with advancing age—reminiscent of the shift of black toward brown with advancing age particularly when a black mouse is heterozygous for brown.

In several cases, the appearance of a germinal mutation affecting color of the hair has also suddenly brought about physiological changes that alter susceptibility or resistance to tumors induced by methylcholanthrene.

It is concluded that there must be some correlation between the induction of germinal mutations by methylcholanthrene affecting body characteristics and the unknown changes in somatic tissue which leads to cancer induced by the same carcinogen.

THE ACTION OF DIAMIDINES AND RELATED COMPOUNDS ON NUCLEOPROTEINS. M. J. KOPAC. (Department of Biology, Washington Square College of Arts and Sciences, New York University, New York 3, N. Y.)

Cellular structures, in particular those containing nucleoproteins, can be modified by a number of chemical agents. Various oncolytic agents (bacterial polysaccharides, quaternary ammonium halides, diphenylethylamines, stilbamidine, and x-radiation) may initiate abnormalities ranging from minor disturbances in nuclear configurations to the complete destruction of the nucleus.

The changes induced in cells by the action of nitrogen mustards frequently cannot be recognized except by careful cytological study or by genetic analysis. These substances, at sub-lethal concentrations, may increase the rate of incidence, in *Drosophila*, of sex-linked lethals—an effect previously obtained only by the application of high frequency radiation.

The so-called mitotic poisons cause still different effects. Colchicine, in most instances, produces a metaphase block. Some of the diphenethylamine derivatives are also mitotic poisons of the colchicine type. Several nitrophenols induce a prophase block in cleaving sea-urchin eggs. Nitrogen mustards have been reported to disturb the interkinetic phase in a variety of cells.

The virus nucleoproteins are also susceptible to chemical agents, especially to those compounds capable of denaturating proteins. Accordingly, detergents, pyridines, picolines, guanidines, and urea have been frequently employed either as destroyers of virus activity, or as tools for the elucidation of virus structure.

The experiments described in this report were designed for two purposes: (1) to measure the effect of selected organic compounds on the structure of various types of nucleoproteins, and (2) to develop and test methods that might be employed later for determining the action of such compounds on living cells.

Experimental.—Changes in protein structure can be followed by measuring denaturation rates. Unfortunately, classical methods were not sufficiently sensitive to measure the denaturation of proteins induced by compounds at low concentrations. A procedure was developed, however, so that protein molecules could be exposed to the simultaneous

action of interfacial forces and one of the compounds maintained at physiologically effective concentrations. In this way, the denaturating effect of one agent could be used to augment a similar action initiated by another agent. For example, with these surface chemical methods, it was previously shown that stilbamidine, at physiologic concentrations, enhanced the interfacial denaturation of various protein molecules. Several other diamidines, however, depressed interfacial denaturation.

In general, three-dimensional protein molecules when exposed to appropriate surface or interfacial forces will unfold and become two-dimensional platelets. Such changes in architecture require the splitting of several critical side-chain linkages. Apparently, some of these linkages can be split by surface forces alone, while others may be split only by the action of chemical agents. When both forces are in operation, the effect can be demonstrated by an increase in surface denaturation of the proteins tested.

On the other hand, other compounds which reinforce side-chain linkages will indicate the effect by a decreased surface denaturation. Several compounds, including fatty acids and certain amino acids, when added to protein solutions can protect such proteins against heat and other denaturating agents. These protecting compounds also appreciably reduce the surface denaturation of protein molecules.

The nucleoproteins (liver nucleoproteins, tobacco mosaic virus nucleoprotein, protamine = nucleate complexes, and a cytoplasmic fraction, rich in mitochondria) were exposed to the simultaneous action of interfacial forces (oil-water interfaces) and one of the chemical agents (at concentrations of 0.001 *M*, or lower). The interfacial denaturation of the nucleoproteins was measured with the semi-automatic, drop-retraction apparatus. The method is fundamentally a micro-adaptation of the procedure developed by Devaux for measuring protein monolayers at oil-water interfaces. The same procedures and apparatus may be used to study oil-cytoplasm interfaces. In this way, the action of chemical agents on cytoplasmic proteins can be determined.

Liver nucleoproteins.—The action of the various compounds already investigated on liver nucleoproteins may be classified into 4 groups:

I. Compounds that augment surface forces by weakening certain side-chain linkages in the protein molecule. Increased interfacial denaturation (stilbamidine, methyl-bis-, ethyl-bis-, and tris-2-chloroethylamine, benzylisothiourea).

II. Compounds that counteract surface forces by strengthening critical side chain linkages. Decreased interfacial denaturation (propamidine, pentamidine, phenamidine, 1,2-di-*p*-anisylethylamine, phenethylpyridinium bromide, bis-amidinomethyldibenzyl).

III. Compounds that neither augment nor counteract surface forces. Unchanged interfacial denaturation (1,2-diphenylethylamine, benzyliminoethylether).

IV. Compounds that simultaneously augment and counteract surface forces by weakening some linkages and strengthening others within the molecule. The net effect depends on the predominance of one action over that of the other. A balance between the two would produce no change in interfacial denaturation. Under such conditions, groups III and IV can be differentiated by testing such compounds in combination with stilbamidine (colchicine, phenethyl-4-picolonium bromide, 2-*p*-dimethylaminostyryl-4-methylpyridine-methobromide, 2-*p*-dimethylaminostyrylpyridine-methobromide, benzylamidine).

Tobacco mosaic virus nucleoprotein.—The surface denaturation of molecules of the virus type requires: (1) dissociation of the large molecules into smaller molecules, and (2) unfolding of the smaller molecules at the interface. Interfacial forces are sufficiently large in some instances to produce both dissociation and unfolding. Agents that only dissociate the large molecules will enhance interfacial denaturation. If these agents also facilitate unfolding, interfacial denaturation would be enhanced to an even higher degree.

Stilbamidine, propamidine, or pentamidine increased the interfacial denaturation of the virus nucleoprotein. The increased denaturation by these compounds was, in all probability, the result of dissociating the giant virus molecule. The experimental data indicate that the subsequent unfolding of the small molecules was induced primarily by surface forces. In no instance has propamidine or pentamidine enhanced interfacial unfolding of protein molecules to any appreciable extent.

On the other hand, phenamidine or bis-amidinomethyldibenzyl appeared to strengthen the virus molecule so that interfacial forces were unable to dissociate the large molecule. Accordingly, interfacial denaturation of the virus nucleoprotein was depressed by these two compounds. Neither bis-amidinomethyldibenzyl nor phenamidine were able to antagonize the action of stilbamidine.

Cytoplasmic proteins.—Stilbamidine enhanced the interfacial denaturation of cytoplasmic proteins, buffered at pH 7.5, while the other diamidines depressed. Propamidine depressed considerably more than phenamidine or bis-amidinomethyldibenzyl. Thus, the latter two diamidines produced similar effects with both virus nucleoproteins and cytoplasmic proteins.

Either stilbamidine or propamidine were equally effective in enhancing the interfacial denaturation of cytoplasmic proteins, buffered at pH 7.9. Colchicine depressed interfacial denaturation, thus producing effects on cytoplasmic proteins similar to those previously described for liver nucleoproteins. Tris-2-chloroethylamine depressed interfacial denaturation of the cytoplasmic proteins, in contrast to its action on liver nucleoproteins.

Combination of chemical agents with stilbamidine.—The striking denaturating action by stilbamidine was not obtained on adding this agent to liver nucleoproteins previously treated with propamidine, pentamidine, or phenamidine. The stilbamidine effect was thereby antagonized and in proportion to the depressing action of the other diamidines tested alone, *e.g.* pentamidine > propamidine > phenamidine. The interfacial denaturation of liver nucleoprotein treated with phenethylpyridinium Br + stilbamidine was nearly identical to that measured with phenamidine + stilbamidine. These data indicate that phenamidine and phenethylpyridinium Br have a similar action.

The interfacial denaturation of liver nucleoproteins in the presence of colchicine + stilbamidine was higher than in the presence of stilbamidine alone. Colchicine, at the same concentration, depressed interfacial denaturation. Phenethyl-4-picolonium Br antagonized stilbamidine slightly, however, subsequent analyses of the denaturation curves suggest that this compound is similar to colchicine.

Some linkages are weakened while others are strengthened in the liver nucleoprotein molecules by either colchicine or phenethyl-4-picolonium Br. If stilbamidine was added with these compounds, interfacial denaturation was enhanced since stilbamidine tends to weaken side-chain linkages. Phenethyl-4-picolonium Br is a stronger depressor than colchicine and accordingly this agent antagonized stilbamidine to a greater degree.

Significance of chemical structures.—Relatively slight changes in structure of an agent may produce considerably different effects on the interfacial denaturation of nucleoproteins. The striking differences between phenethylpyridinium Br and phenethyl-4-picolonium Br show that a single *p*-methyl group can modify the action of a compound on liver nucleoproteins.

All aromatic diamidines, with the exception of stilbamidine, depressed interfacial denaturation of liver nucleoproteins. Liver nucleoprotein molecules were fortified against interfacial forces by bis-amidinomethyldibenzyl and weakened by stilbamidine. When the two agents were present simultaneously, the weakening action of stilbamidine was almost entirely antagonized. The other diamidines also antagonized the action of stilbamidine and in proportion to their ability to antagonize interfacial forces.

Many oncolytic compounds induce morphological or functional changes in tumor cells especially in those structures that contain nucleoproteins. These agents also produced clear-cut effects on the interfacial denaturation of liver nucleoproteins, virus nucleoprotein, protamine = nucleate complexes, or of cytoplasmic proteins.

Any change in interfacial denaturation implies some fundamental alteration in the structure of such nucleoproteins and these alterations may, in many instances, be of sufficient magnitude to affect the functional activity of those cells in which corresponding changes in nucleoprotein structure might occur. The ability of certain compounds to bind side-chain linkages and at the same time weaken others may explain, in part, the action of mitotic poisons.

Nucleoproteins may even be dissociated by a chemical agent, providing the protein moiety is, at least partially denatured. In complex nucleoproteins, denaturation of the protein moiety is a prerequisite for the separation of nucleic acid from the protein molecule.

Thus, nucleoproteins can be inactivated in two ways: (1) the nucleic acid may be irreversibly dissociated from the protein moiety, or (2) critical intramolecular structure of the nucleoprotein may be blocked by strengthening side-chain linkages. In either event, such molecules must become less capable of performing essential functions.

There is surface chemical evidence to indicate that specificity of action might be further enhanced by employing a combination of agents, in which the individual properties are mutually modified. For example, the combination of stilbamidine + colchicine was different in its action on nucleoproteins than that obtained by the individual action of either agent. Similarly, the effects produced by stilbamidine + phenethyl-4-picolonium Br or by stilbamidine + diphenylethylamine differed strikingly from those produced by any one of the agents tested individually. Similar combinations of chemical agents should, therefore, be tested on living cells.

SOME CHEMICAL CHANGES INDUCED BY METHYLCHOLANTHRENE IN THE TRANSFORMATION OF MOUSE EPIDERMIS TO SQUAMOUS CELL CARCINOMA.

C. CARRUTHERS and V. SUNTZEFF. (Research Department, The Barnard Free Skin and Cancer Hospital, St. Louis 3, and the Department of Anatomy, Washington University Medical School, St. Louis 10, Missouri.)

The research program at this hospital on some of the chemical, histological, and physical changes induced in the epidermis of mice undergoing carcinogenesis by methylcholanthrene has led to the accumulation of considerable data on the chemical composition of normal, methylcholanthrene-treated (hyperplastic) epidermis, and transplantable squamous cell carcinoma of mice, and also some information on the mineral composition of human epidermis.

For the various analyses, the epidermis was separated from the dermis at 50° C. on a warm plate by the procedure of Baumberger, Suntzeff, and Cowdry (1), or at room temperature by a recently devised method. Since only small amounts of epidermis were obtained from normal mice, it was necessary to devise microchemical methods for the quantitative determination of the minerals, vitamins, and lipids.

Using nucleoprotein phosphorus as a basis of reference, the potassium, calcium, magnesium, iron, copper, and zinc contents of normal epidermis, hyperplastic epidermis, and of a transplantable squamous cell carcinoma were determined. While significant decreases in the calcium, iron, copper, and zinc nucleoprotein phosphorus ratios were observed as a result of the application of 1 to 3 paintings of the carcinogen, the potassium, sodium, magnesium, and ascorbic acid ratios showed no perceptible change. Moreover, the decrease in the heavy metal and calcium ratios so manifestly evident throughout hyperplasia was even more spectacular with the onset of carcinoma. On the other hand, the ascorbic acid nucleoprotein phosphorus ratio remained unchanged throughout the hyperplastic and carcinomatous stages, while the sodium, magnesium, and potassium ratios decreased slightly in the tissues of squamous cell carcinoma.

In this connection, analyses were also made of the mineral composition of human epidermis (for purposes of comparison) and it was discovered that decreases occurred in the calcium, copper, and zinc contents in squamous cell carcinoma strikingly similar to those detected in the comparable stage of the mouse. These experiments also revealed that normal human and hyperplastic epidermis of the mouse agree well with respect to their mineral composition, which is in conformity with their somewhat similar morphology.

Determination of the water content of the epidermis undergoing carcinogenesis established the fact that the amount of water increased from 60 per cent in the normal to an average value of 66 per cent in the hyperplastic stage, and rose to nearly 82 per cent in the transplantable carcinoma.

Investigations on the epidermal content of choline, biotin, *p*-aminobenzoic acid, inositol, and pyridoxine by Tatum and his group (4) using mutant strains of *Neurospora* demonstrated that only biotin was significantly altered during carcinogenesis. This vitamin decreased to 64 per cent of the normal.

Wicks and Suntzeff (5, 6) also showed that the total lipid- and total cholesterol-protein-nitrogen ratios were about 50 per cent less than normal in hyperplastic epidermis. The decrease in the total lipid was found to synchronize with the disappearance of the sebaceous glands. The relationship of the latter structures to carcinogenesis has been investigated by Simpson and Cramer (3), and is briefly discussed.

Experiments were also conducted with a view to elicit information concerning the influence of age on the response of carcinogen in relation to epidermal calcium and total lipid content. Young and old mice of the Swiss and New Buffalo strains were used for the purpose. The results showed conclusively the absence of any correlation between the decrease in the content of these substances and the response to the carcinogen.

The activities of succinic dehydrogenase and cytochrome oxidase in epidermal carcinogenesis were followed by employing the procedure of Schneider and Potter (2). Our study showed that the activity of succinic dehydrogenase remained the same both in the normal untreated epidermis and in the hyperplastic epidermis, but increased significantly in the carcinoma. On the other hand, the activity of cytochrome oxidase in the late hyperplastic epidermis was twice that of the normal, but the value for its activity in the carcinoma was less than that of hyperplastic epidermis, but greater than that of the normal.

Finally, an attempt was made to correlate the chemical changes induced by the carcinogen with some of the morphological alterations.

REFERENCES.

1. Baumberger, J. P., Suntzeff, V., and Cowdry, E. V. Methods for the Separation of Epidermis from Dermis and Some Physiologic and Chemical Properties of Isolated Epidermis. J. Nat. Cancer Inst., **2**:413-423. 1942.
2. Schneider, W. C., and Potter, V. R. The Assay of Animal Tissues for Respiratory Enzymes II. Succinic Dehydrogenase and Cytochrome Oxidase. J. Biol. Chem., **149**:217-227. 1943.
3. Simpson, W. L., and Cramer, W. Sebaceous Glands and Experimental Skin Carcinogenesis in Mice. Cancer Research, **3**:515-518. 1943.
4. Tatum, E. L., Ritchey, M. G., Cowdry, E. V., and Wicks, L. F. Vitamin Content of Mouse Epidermis During Methylcholanthrene Carcinogenesis. I. Biotin, Choline, Inositol, p-Aminobenzoic Acid, and Pyridoxine. J. Biol. Chem., **163**:675-682. 1946.
5. Wicks, L. F., and Suntzeff, V. Reduction of Total Lipid-Protein-Nitrogen Ratio of Mouse Epidermis by a Single Application of Methylcholanthrene. J. Nat. Cancer Inst., **3**:221-226. 1942.
6. Wicks, L. F., and Suntzeff, V. Changes in Epidermal Cholesterol During Methylcholanthrene Carcinogenesis in Mice. Cancer Research, **5**:464-468. 1945.

THE SIGNIFICANCE OF INDUSTRIAL CANCER IN THE CANCER PROBLEM.

W. C. HUEPER. (Warner Institute for Therapeutic Research, New York, N. Y.)

In view of the known differences existing in the susceptibility and reactivity of various species to the numerous recognized physical and chemical carcinogenic agents, industrial cancers possess the great advantage over experimental cancers in animals in that they permit the direct study of many of the most important and fundamental aspects of cancer in the human organism. The considerable rise in the absolute number and in different types of occupational cancers paralleling the enormous growth of modern industrial developments reflects an important biological maladjustment of civilized man to his new artificial industrial environment and represents a striking illustration of the momentous rôle which exogenous environmental agents apparently play in the causation of cancer in general. There are good reasons for assuming that the majority of occupational carcinogenic agents is still unknown, and this applies especially to those occupational carcinogens which have a low potency and therefore produce a low incidence of cancer among the individuals exposed. This conclusion is supported by evidence obtained during recent years which makes it likely that new industrial carcinogenic agents and heretofore unknown types of occupational cancers will be discovered in the next few years in American industries. When these facts and their implications are properly and fully appreciated, occupational cancers are bound to become a public health problem of the first order, which deserves serious and extensive investigation.

The existence of wide differences in the physical and chemical properties of the various established or suspected occupational carcinogens suggests that these agents do not exert their specific pathogenic action through the same mechanism. Some of them seem to cause a cancerous cellular transformation by acting directly either in their original form or as metabolites or conjugates upon the cellular substrate. Others may elicit such an effect by changing some normal chemical constituent of the cells or tissue fluids in such a way that it becomes endowed with carcinogenic properties. A third group of occupational carcinogenic agents may display such an action by producing functional disturbances in certain organs, such as the liver, adrenal glands, pituitary gland and gonads leading to the endogenous generation of carcinogenic substances.

Inasmuch as the great prevalence of industrial cancers in males is the result of an exposure factor and is evidently not sex-conditioned, it is suggested that environmental carcinogenic factors may be responsible for the greater frequency of several cryptogenetic cancers (lung, larynx, gastrointestinal tract, skin) among males than among females.

Observations made in connection with occupational cancers show clearly that a senescence factor is not involved in the production of these tumors, which have been seen in individuals between the ages of 10 to 80 years. The age at onset of exposure together with its duration and intensity controls the manifestation age of occupational cancers.

There is no concrete and valid evidence indicating that a genetic hereditary predisposition plays any important rôle in the production and incidence of occupational cancers. The intensity, type and duration of exposure to, and the potency of the carcinogenic agents involved appear to be the main factors in determining the susceptibility of the individual and the length of the latent period.

The rôle of the various procarcinogenic, anticarcinogenic, procarcinotic and anticarcinotic factors is evidently not a fixed one, but varies with the type and contact of the carcinogenic agent involved and the intensity of its action.

Although the United States has been for several decades the leading industrialized country, only very incomplete data as to the actual number and variety of occupational cancers, their causes, and the agents and operations responsible for their development are available, because these industrial diseases are not reportable in most of the states and because American industries have been reluctant to place their cases of occupational cancer on record. By the end of 1945 only 32 states had passed some form of compensation law covering industrial diseases in general. Since the majority of such laws either contain time limitation clauses as to the filing of compensation claims or apply only to a limited and well defined number of hazardous agents or operations, they provide inadequate or defective coverage against industrial cancer hazards. No competent and extensive investigations exist in regard to the possible rôle that industrial or related exogenous agents might play in the causation of some of the large numbers of cancers of the cryptogenetic type. A comprehensive and thorough survey of all industries producing or handling known or suspected carcinogenic agents, including

those occurring in the form of contaminants, by-products or waste products, would furnish a sound basis for the development of effective measures controlling the industrial cancer hazards of this country.

CARCINOGENIC ACTION OF SOME SUBSTANCES WHICH MAY BE A PROBLEM IN CERTAIN FUTURE INDUSTRIES. AUSTIN M. BRUES, HERMANN LISCO, and MIRIAM P. FINKEL. (Argonne National Laboratory, University of Chicago, Chicago 80, Illinois.)

In the course of manufacturing plutonium in a chain-reacting pile, products of uranium fission are evolved. These atomic split products are largely radioactive and must be separated from the pile uranium as part of the plutonium separation process.

We have investigated the late or chronic effects of exposure to certain fission products and plutonium. The fission products, since they occupy the middle of the periodic table, include most of the rare earths, also iodine. barium and strontium. Experiments were set up to observe the effects of radioactive Sr^{89}, Ce^{144}-Pr^{144}, Y^{91}, and plutonium. Radium was also studied for comparison with earlier human and animal work. We used Sprague-Dawley rats, Carworth CF-1 and ABC hybrid mice and a few C58 mice, as well as rabbits and dogs. All animals received complete autopsy, terminal blood study, and roentgenograms. This is a progress report.

Sr^{89}, with a 55-day half-life and a tendency to concentrate in bone, has no visible effect on mammary tumor incidence and a slight positive effect on lymphoma incidence. It is a producer par excellence of bone tumors. Analysis of bone tumor incidence in a series of over 3,000 mice, 400 to 500 days after treatment, shows that expectance of tumor development is approximately proportional to dose and to time after the onset of a "latent period," but that the latent period itself is a function of dose which increases only gradually (perhaps logarithmically) with decreasing dose. Such a scheme is shown to fit, as well as can be determined, the existing human radium data. It appears that age of the animal at the time treatment is administered has little effect on latent period or tumor expectancy, even when the isotope is absorbed by the growing fetus.

Radium, in addition to bone tumor induction, produces heavy calcification in the media of the larger arteries and in certain other sites.

Radioactive cerium—presyodymium, which gives an initial high liver concentration, causes atrophy of this organ except in the marginal areas, where ionization is less and regeneration can proceed. Bone sarcoma is also produced.

Yttrium^{91}, which was fed by stomach tube, is virtually unabsorbed. One-tenth of the acute lethal dose can be fed daily for 3 months with no measurable detriment. Animals surviving this treatment, as well as those surviving a single acute dose of 20 to 30 mc. per kgm., show after several months a variety of intestinal lesions with obstruction.

Plutonium (Pu^{239}), when injected intravenously, has its highest immediate concentration in liver and spleen, and is later translocated to bone. Acute plutonism is similar to acute total body radiation sickness, with the additions of splenic atrophy and gross liver changes. Chronic plutonism involves greying of hair, progressive liver damage, and bone sarcoma. Following subcutaneous injection of 1 μgm. of plutonium, local fibrosarcomas may appear within a year. Local epilations, ulceration, keratosis, and spontaneous amputation are also seen.

THE CARCINOGENIC EFFECT OF PILE RADIATIONS. P. S. HENSHAW, E. F. RILEY and G. E. STAPLETON. (Clinton Laboratories, Oak Ridge, Tennessee.)

A variety of high energy radiations (fast and slow neutrons, gamma rays, beta rays, etc.) are associated with the uranium chain reaction in a pile reactor. All of these radiations are known to cause deleterious biological effects and hence constitute health hazards.

In an attempt to understand more fully the types of injury induced, and at the same time obtain information on threshold and tolerance levels, numerous animal experiments were carried out. Four strains of mice (CF_1, ABC, A, C58) and one strain of rats (Sprague-Dawley) were the main types of test material. The different radiations were obtained by means of selective filtration, secondary emitters and radioactive isotopes. Single massive dose treatments as well as small daily treatments were administered. The radiations used, with the exception of beta rays, were of the penetrating type, and for the most part were distributed throughout the animal bodies.

Results revealed that the length of life varied inversely with the amount of exposure, and further that the amount of effect varied directly with the concentration of ions in space and time. The terminal effects obtained when penetrating radiations were used were mainly of two types: generalized atrophy, and neoplasia of the hemopoietic organs. The atrophy was attended by lowered leukocyte level, emaciation, loss of weight, and premature death. The neoplasms seen consisted mainly of mediastinal lymphomatoses. The incidence of the latter was raised from less than 15 per cent in the controls to more than 60 per cent in certain of the experimental groups.

When non-penetrating radiations were used the changes were limited mainly to the skin. Several months after single massive doses skin abnormalities of every type were present. In animals that rarely show skin lesions of any type, the incidence of carcinoma was raised to 100 per cent and loci per animal were as great as 50 to 100.

The relative effectiveness of fast neutrons when compared with gamma rays appeared to be definitely greater when late modifications were considered rather than acute. Threshold levels of daily exposure, so far as shortening of life is concerned, were found to be in the range of 1 r of gamma rays, and something well less than 0.1 r.

ETIOLOGIC STUDIES IN HODGKIN'S DISEASE. HERMAN A. HOSTER. (The Division of Cancer Research, The Ohio State University, Columbus, Ohio.)

An introductory reference was made to the distribution of Hodgkin's disease; the significance of tubercle bacilli and *Brucella* in Hodgkin's tissue and body fluids; the age distribution of patients with Hodgkin's disease; the prognostic importance of the sedimentation rate, lymphopenia, and anemia; the absence of pain in early Hodgkin's disease; and the selective preference of the disease for specific tissue systems.

Observations following inoculation of tissue culture cells with cell-free material from Hodgkin's disease, lymphomatosis, and control tissue extracts and body fluids were described. Embryo spleen cells in tissue culture, stained with Seller's stain, were used routinely to demonstrate the presence or absence of fuchsinophilic cytoplasmic inclu-

sions. Sources of embryo spleen were the chicken, duck, and guinea pig. All preparations of fresh, frozen, or lyophilized Hodgkin's tissue, lymphomatosis, and control tissues were minced and centrifuged for 30 minutes at 4,000 r.p.m. The supernatant fluid obtained was used as the source of inoculum before and after differential low gravity and ultracentrifugation, ultrafiltration, and dilution to one part in 2 billion. Cyto-stimulating and cytolytic changes were noted following inoculation of tissue cultures with cell-free material of Hodgkin's disease and lymphomatosis. These changes were not observed in cultures inoculated with control tissues and body fluids. Inocula composed of the resuspended sediment following high gravity centrifugation produced in tissue cultures an increase in cytostimulation and the number of inclusion bodies.

Fuchsinophilic intracytoplasmic inclusions were found not only in tissue cultures inoculated with material from Hodgkin's disease and lymphomatosis, but also were noted in some control preparations containing either chicken or duck constituents only. Investigation of this phenomenon resulted in the finding that chickens and ducks, from which the plasma and embryos had been obtained, were infected with lymphomatosis. Because of this potential source of contamination, adult and embryo chicken and duck materials were considered unsuitable for further use in tissue cultures. Therefore homologous guinea pig substrate and nutrient medium were adopted.

Inactivation of the agents of Hodgkin's disease and lymphomatosis by ultraviolet light was described.

The third phase of the work deals with clinical observations made by the author and his associates. Under the direction of Dr. Perk Lee Davis of the Women's Medical College of Pennsylvania, two patients with Hodgkin's disease received intramuscular injections of 10 cc. of pleural fluid obtained from a terminal case of Hodgkin's disease of the thoracic type. The donor also received an identical injection of his own fluid. At the end of 48 to 72 hours all 3 recipients developed a generalized maculopapular eruption with severe itching. Most of the lesions soon disappeared, but a few still remained; these enlarged, and some showed a central necrosis between the fourth and seventh days following the injections. These lesions varied in size from 0.2 to 2.0 cm. and did not respond to treatment with chemotherapeutc agents applied locally. Examination of biopsy material obtained from the skin lesions revealed the typical Sternberg-Reed cells (Hodgkin's cells) in all cases. Following therapeutic doses of x-rays, these lesions disappeared leaving brown pigmented areas, but the downhill course of the patients appeared to be accelerated following the injections.

Lesions similar to, but less acute than, those just described have been observed by the author in several patients with Hodgkin's disease:

(a) Multiple skin lesions occurring immediately after a series of five daily x-ray treatments to a greatly enlarged spleen were seen in case of H. O., a 37 year old white male with acute Hodgkin's disease.

(b) M. H., a 34 year old white female, developed multiple skin lesions of the back 48 hours following the subcutaneous inoculation of an extract of Hodgkin's tissue that had been inactivated by ultraviolet irradiation.

(c) The author has observed 4 cases of Hodgkin's disease that exhibited skin lesions of spontaneous origin similar to those described above by Dr. Davis. One of the author's associates, Dr. Anthony Rottino, of St. Vincent's Hospital in New York City, has investigated and made biopsies of 2 similar cases.

TISSUE CULTURE STUDIES OF LYMPH NODES OF HODGKIN'S DISEASE.

C. G. GRAND. (New York University Washington Square College, New York 3, and St. Vincent's Hospital, New York, N. Y.)

This report is based on the study, including tissue culture, of lymph nodes from 75 cases of early and late stages of Hodgkin's disease. As controls, normal lymph nodes were used, also lymphosarcomas, leukemias, metastatic carcinomas and lymphadenitis.

The tissue cultures were maintained for periods varying from a few days to several weeks. After 72 hours of incubation, the cultures of the Hodgkin's disease showed, on the periphery of the explant, large multinucleated giant cells of the endothelial type which resembled Reed-Sternberg cells. They were absent in cultures of other lymphomas and of normal nodes.

Brilliant cresyl blue, as a vital stain for virus cell inclusions, stained the granules of the central body of these cells from red to purple within 10 to 15 minutes. Similarly stained inclusions also were found in macrophages, lymphocytes, and in many cases fibrocytes. Seller's stain was also used. This colored the inclusions a bright red, while the nucleus and regular cell granules were deep blue.

The outgrowing cells of the Hodgkin's disease cultures were seen to disintegrate in such a manner as to indicate some sort of infection. This is not seen in cultures of true neoplasms. The time for complete disintegration of a culture depends on the number of Reed-Sternberg cells and the number of cells containing inclusion bodies. The time may be from 1 to 49 days before all the cells in the culture have cystolysed and the medium liquefied.

Supernatant fluid was removed from cultures of Hodgkin's tissues grown in Carrel flasks and was centrifuged at 2,000 r.p.m. for half an hour. When this cell-free fluid was added to cultures of normal human lymph node, chick spleen, mouse spleen and lymph node, the lymphocytes and macrophages showed specific cell inclusions within vacuoles after 3 days. Serial passages in tissue culture of the supernatant fluid showed an increase in potency so that at the seventh passage, the time for complete disintegration of the cultures was cut from 10 or 15 to 3 days. There was also an increase in the number of cell inclusions with the successive passages. Supernatant fluid from cultures of normal human lymph nodes and lymphomas other than Hodgkin's disease gave negative results.

Concentration of the agent was done by fractionation centrifugation and was found to be very active when added to tissue cultures of chick and mouse tissues when diluted 1:1,000.

This material will not pass bacterial filters except under certain conditions. It was found to keep its activity for 2 years at 4° C. if the purified material is suspended in .05 per cent serum hydrolysate at pH 7.0 or if suspended in inactivated rabbit serum.

THE EFFECT OF NITROGEN MUSTARDS ON PROLIFERATING EMBRYONIC TISSUES.

D. BODENSTEIN. (Medical Division, Edgewood Arsenal, Maryland.)

Embryos of *Amblystoma punctatum* (tail-bud-stages) were exposed for 45 minutes to a 0.001 per cent solution of the nitrogen mustard compound methyl-bis (betachloroethyl) amine hydrochloride.

The agent affects selectivity centers of proliferation and leaves differentiating regions unaffected. Within a short

time of the exposure (about 2 days) all mitotic activity is abolished. The affected mitotic cells exhibit clearly two distinct reactions. They either break down rather rapidly into chromophilic fragments or increase strikingly in size. The nuclei of these large cells may fragment in later development, while some of them seem to recover. In organs where the processes of proliferation and differentiation occur in different cells, as in the nervous system for example, enlargement and early breakdown of the mitotic cells occur side by side. In the embryonic gut, however, where differentiation and proliferation occur within the same cell, only cell enlargement occurs, while at the same time cellular differentiation continues unhindered. This results in the formation of giant cells which apparently function normally. It was found that the cells showing the enlargement reaction are characteristically cells which in later development make up proliferating regions of the embryo, while the cells exhibiting the early breakdown reaction without enlargement are those whose mitotic activity is limited to early embryonic development. Consequently fewer and fewer broken cells of this type are found as the age of the animals at exposure increases, because more and more of these mitotic cells have entered their differentiation phase and are hence not affected.

By its selective action in showing up regions of proliferation the agent is an exceedingly useful tool for the study of growth patterns. The agent's specific ability to arrest mitotic activity without interfering with differentiation allows us to control experimentally the size of organs and gives us a new approach to the problems of growth control.

EXPERIMENTAL OBSERVATIONS ON THE EFFECTS OF THE NITROGEN MUSTARDS ON NEOPLASTIC TISSUES

D. A. KARNOFSKY. J. H. BURCHENAL, R. A. ORMSBEE, I. CORNMAN. and C. P. RHOADS. (Sloan-Kettering Institute, New York 21, N. Y.)

The effects of the nitrogen mustards $N(C_2H_4Cl)_3$ (called HN3), $CH_3N(C_2H_4Cl)_2$ (called HN2), $C_2H_5N(C_2H_4Cl)_2$ (called HN1) and $\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>\!\!CHN(C_2H_4Cl)_2$, used in all cases as hydrochloride salts, have been studied on normal animals and tissues, and then assayed for chemotherapeutic activity against various types of neoplastic tissues under several conditions: Leukemia in mice, mouse sarcoma 180 growing on the chick chorioallantoic membrane and sarcoma 180, adenocarcinoma 1025 and lung carcinoma MA 387 in roller-tube cultures.

In normal animals it is found that certain tissues, including the hematopoietic and lymphatic tissues and the intestinal epithelium, are unusually sensitive to the action of these compounds. It may be significant that the sensitive tissues are those which possess relatively high rates of cell division. HN2 and HN3, the only ones tested, on intravenous injection into animals have a very rapid and presumably direct action on the affected tissues. In many respects the sulfur and nitrogen mustards are found to resemble x-rays in their action; for example, in their toxicological effects on animals, their inhibiting action on cell division, and in their ability to produce mutations in *Drosophila* (2) and *Neurospora* (3).

The additive lethal effects in mice of HN2 and x-rays vary with the sequence of administration. If 80 per cent of a lethal dose of x-ray is followed an hour later by 80 per cent of a lethal dose of HN2, the lethal action of the two agents is only slightly additive. If the sequence is reversed, and the same dose of HN2 is given 1 hour before the x-rays, an additive, much greater lethal effect results.

Four compounds, the methyl-bis, ethyl-bis, isopropyl-bis and the tris-(β-chloroethyl)amine were tested against 1 myeloid and 3 lymphoid leukemias in mice. The mice used were of a highly inbred stock Afb in which transmitted leukemias of the 4 strains used take in approximately 100 per cent. The experimental procedure was as follows: a leukemic mouse with enlarged spleen and nodes was weighed and injected intravenously with a given mgm./kgm. dose of the compound to be tested dissolved in saline. One hour later (less in the higher doses where toxic effects often killed the mouse in from 5 to 50 minutes) the mouse was killed, the spleen removed, and the splenic brei injected subcutaneously into 4 young mice of the Afb stock as a bioassay. Sections of liver, spleen, and kidney of the donor mouse were made. All mice used for bioassay were then watched for development of leukemia, and in all questionable cases sections were made. It has been our experience that large doses of the nitrogen mustards, even when not completely cytocidal to leukemic cells, so affect them that the incubation period in the bioassay animal may be much prolonged over the normal time.

The problem of how long the nitrogen mustard should stay in the live animal in order to have a maximal effect on the leukemic cells of the spleen is unsolved. Frequently the animal died rapidly and we arbitrarily set 7 minutes survival time for the donor animal as necessary for a valid bioassay.

From the preliminary data, it would appear that for the 4 strains of leukemia tested the cytocidal dose of tris-(β-chloroethyl)-amine varies from 7 to 22 times the LD50 or 10 to 33 mgm./kgm. The methyl-bis compound inactivates the leukemic cells at 8 to 12 times the LD50 or 33 to 75 mgm./kgm. With the ethyl-bis compound, only in the Af9417 leukemia were there any consistent results and there the cytocidal effect was manifest at 22 times the LD50 or 33 mgm./kgm. With the isopropyl-bis(β-chloroethyl)-amine, a dosage of 37 times LD50 or 50 mgm./kgm. inactivated the cells of 3 strains of leukemia in some experiments.

It appears from this experiment that with the tris- and the methyl-bis compounds there is a definite cytocidal dose for leukemic cells. With the ethyl and isopropyl analogs, the evidence of cytocidal activity is not clear even at much higher multiples of the LD50. If this is borne out in the finished experiments, it would seem to indicate that the former compounds are more likely to be of value clinically than are the latter.

The lethal dose of HN3 in fertile chick eggs is almost directly proportional to the mass of the embryo; the LD50 by injection into the yolk sac is about 0.015 mgm./egg in the 3-day chick and about 0.5 mgm/egg in the 12-day chick. Anomalies of the beak and legs have been produced in 3-day-old chicks surviving LD50 doses of HN3, but this has not occurred in the older eggs. A similar effect has been produced with the sulfonamides (1). In 12 days eggs with actively growing sarcoma 180 tumors on their chorioallantoic membrane, a dose of HN3 which is not lethal to the egg will stop the growth of the sarcoma 180, render it incapable of growth when transplanted into mice, and produce, as shown by histological examination, dissolution of a majority of the sarcoma cells and the appearance of remarkable giant cells containing large ab-

normal nuclei, occasionally multilobed or fragmented. The appearance and location of these giant cells strongly suggest that they stem from the sarcoma cells. No significant change in the chick membranes or vascular tissues was observed. The LD50 of HN3 for the tumor in the egg is in the range of 0.05 to 0.10 mgm./egg, when injected at 12 days, about 1/5 to 1/10 of the LD50 for the chick embryo. Preliminary experiments designed to show the speed of the lethal action of HN3 in such egg-tumor preparations indicate that the tumor can be fatally damaged within less than 30 minutes (the shortest period studied) by 0.2 mgm./egg of HN3.

Using the roller tube technic, cultures of sarcoma 180, lung carcinoma Ma387, and normal mouse epithelium were exposed to various concentrations of $N(C_2H_5Cl)_3$ dissolved in the nutrient medium. The results of these experiments up to the present time indicate that certain tumors appear to be more susceptible than others to the action of this compound. Sarcoma 180 cells have consistently been killed at concentrations of 20 mgm./1 after 24 hours exposure as indicated by their failure to cause tumors when transplanted into susceptible mice. MA387, however, has in some cases survived concentrations as high as 80 mgm./1.

There was suggestive evidence that the tumor cells were somewhat more sensitive to concentrations of HN3 than were the corresponding normal cells. At concentrations of up to 80 mgm./1, the deleterious effects on normal cells appeared less distinct than on tumor cells. At 40 mgm./1, Ma387 and sarcoma 180 cells were visibly affected although not disintegrated, whereas normal cells appeared unaffected. At 20 mgm./1, both normal and tumor cells looked normal cytologically, but those of sarcoma 180 were apparently rendered nonviable as evidenced by negative bioassay results.

While these lethal concentrations will have to be more precisely defined by further experiments, it is interesting to note the difference in susceptibility of the tumors employed and that tumor cells could be consistently rendered nonviable by concentrations which caused little visible damage to the cells involved.

The clinical evaluation of the methyl-bis and tris-(β-chloroethyl)amine hydrochlorides is in progress. In addition to varying the dosage level, the effect of these drugs on various neoplasms not connected with the blood-forming organs is being evaluated. Neuroblastoma, Ewing's tumor, glioblastoma, and malignant melanoma are under treatment at the present time, but it is still too early to note any definite results.

It is to be re-emphasized here that this is in the nature of a preliminary report and that much work remains to be done before these data can be considered definitive.

REFERENCES

1. Ancel, P., and Lallemand, S. Sur une malformation du bec et des membres obtenue chez l'embryon de Poulet, á l'aide de sulfamides. Compt. rend. Soc. de Biol., **136**:255-256. 1942.
2. Auerbach, C., and Robson, J. M. Chemical Production of Mutations. Nature, **157**:302. 1946.
3. Horowitz, N. H., Houlahan, M. B., Hungate, M. G., and Wright, B. Mustard Gas Mutations in *Neurospora*. Science, **104**:233-234. 1946.

THE CLINICAL APPLICATION OF A NITROGEN MUSTARD COMPOUND METHYL BIS (β CHLOROETHYL) AMINE TO THE TREATMENT OF NEOPLASTIC DISORDERS OF THE HEMOPOIETIC SYSTEM.*

CHARLES L. SPURR, LEON O. JACOBSON, TAYLOR R. SMITH, and E. S. GUZMAN BARRON. (Dept. of Medicine, University of Chicago, Chicago, Illinois.)

Gilman, Goodman and their co-workers (1) were the first to study the effects of a nitrogen mustard compound on human neoplasia. They studied the pharmacological and clinical effects of tris-(β-chloroethyl) amine administered intravenously in 7 terminal cases. Independently in March, 1943, Jacobson and his group (2, 3), undertook a similar clinical study in an attempt to evaluate another nitrogen mustard; namely, methyl-bis (β-chloroethyl) amine as a therapeutic agent. An unselected group of 59 cases of neoplastic and allied disorders of the hemopoietic system have been studied.

Methyl-bis (β-chloroethyl) amine which is highly cytotoxic is administered intravenously in a dose of 0.1 mgm. per kgm. of body weight diluted in approximately 100 cc. of normal saline on consecutive days in series of 2 to 6 injections. The toxic reactions observed after the administration of this compound have been nausea and vomiting, phlebothrombosis, varying degrees of destruction of hemopoietic tissues and consequent depression of the hematological constituents of the peripheral blood. Nausea and vomiting which are partially central and local in origin usually follow each dose within 3 hours and persist for a similar period. Phlebothrombosis occurs only if the drug is not sufficiently dilute when injected or delayed by obstructing masses in the veins used for the administration.

Evaluation of the reaction of the hemopoietic system to the therapeutic application of the methyl-bis (β-chloroethyl) amine demonstrated that, following each series, a characteristic lymphopenia became apparent within 48 hours. Recovery of the lymphoctyes usually begins in the second week following treatment.

A neutropenia develops in the third week and is usually of about one week's duration. There is also a prompt but variable thrombocytopenia during the first 3 weeks. This is occasionally sufficient to produce an increase in the bleeding time but only on 3 occasions have petechiae appeared.

A series of tri-weekly sternal marrow aspirations were made on 5 cases over a period of 6 weeks after treatment. These show a depression of the total nucleated cell count from an average of 100,000 per cu. mm. to 6,400 per cu. mm. on the 21st day and a sharp recovery to 65,000 per cu. mm. in the fourth week. There is a slight depression in the blast forms and promyelocytes during the first week and a moderate increase in the second week. The myelocytes and metamyelocytes decline through the first 2 weeks and regenerate to a peak in the third and fourth weeks respectively. The percentage of polymorphonuclear cells increases in the first 2 weeks and declines sharply in the third week, regenerating to a peak in the fifth week. There is also a depression in the erythroid series for 2 weeks with orderly regenerative peaks of proerythroblast and basophilic erythroblasts in the third week and of the orthochromatic erythroblast in the sixth week. Many giant forms of the granular series are seen in the peripheral blood especially during the first week after administration of the drug. Infections and other clinical manifestations usually attributed to severe leukopenias were conspicuously rare. In the one instance in which an infec-

* This project has been supported in part by a grant from the Committee on Growth of the American Cancer Society.

tion occurred it was successfully controlled by penicillin therapy.

The reaction of the peripheral blood and the clinical response served as the principal guides in evaluating the most efficacious total dose per course of treatment. In a series of 79 courses the severity of the depression of the peripheral blood increased with the number of consecutive injections per course. There was a fall of hemoglobin and erythrocyte values from 0.7 gm. per cent and 140,000 per cu. mm. respectively following 2 injections (average total dose 12 mgm.) to 1.6 gm. per cent and 460,000 per cu. mm. respectively after 6 injections (average dose 36 mgm.). An increasingly severe decline in leukocytes was also noted. The average percentage of fall in the total leukocyte count increased from 35 per cent to a plateau at 65 per cent and 67 per cent as the courses were increased from 2 to 4 and 6 injections. An irregular increase in the incidence of leukopenias of less than 3,000 cells per cu. mm. developed as the courses were prolonged; however, there was a sharp increase in the severity and duration of leukopenias with total doses above a 25 to 30 mgm. level or with 4 or more injections.

The possibility that this hemotoxic material may have a cumulative effect with repeated doses and eventually lead to a permanent hypoplastic reaction in the bone marrow has been assessed by grouping 58 courses according to the sum total dose administered in 50 mgm. steps from 50 to 250 mgm. The reaction of the peripheral blood in each group as judged by the average pretreatment and post-treatment leukocytic counts shows no remarkable trend. However, the percentage incidence of leukopenias increases from 40 per cent in the 0 to 50 mgm. group to 86 per cent in the group with a cumulated dose of 200 to 250 mgm. This is the only indication that the hemopoietic system may become more sensitive to the compound as the courses of treatment are repeated. Two factors tend to contradict this: (a) the leukocytes return to normal range following each course, and (b) the cases in this group are of longer duration and several had leukopenia prior to initiating treatment. It is suggested that a cumulated dose of 600 mgm. or more will be necessary before an unequivocal answer may be made to this question.

The clinical response to methyl-bis (β-chlorethyl) amine hydrochloride in 29 cases of Hodgkin's disease observed over a period of 3 to 36 months shows instances in which the various symptoms of the disease have been controlled. In this group of cases 94 per cent of 120 courses of treatment produced clinically significant remissions. Among the 8 cases that failed to respond to treatment, 5 were in a terminal phase of their disease, 2 had advanced disease and were no longer responsive to roentgen therapy and 1 had a remission from fever of only 3 weeks. Of particular interest are 4 cases which had been considered "x-ray resistant." This group has responded repeatedly to courses of nitrogen mustard. The remissions induced in this group of patients with Hodgkin's disease vary from 0 to 10 months, although the length of remission increases with the total dose per course. A course of four injections of 25 to 30 mgm. was selected as optimum because of the plateau in the severity of leukopenia at this level. There is a slight decrease in the average remission with repeated courses; however, the number of cases cumulating doses above 150 mgm. is too small to evaluate this question. Two cases have appeared to develop a tolerance or resistance to the material.

A group of 9 cases of chronic lymphatic leukemia has shown remissions of 2 to 24 months. This variation depends largely upon the activity of the disease. Although each case responded to the initial course, the group survival is poorer than in Hodgkin's disease. Five patients have died within 18 months after the initial treatment from advancing disease or its complications.

Six cases of lymphosarcoma have been treated with methyl-bis (β-chloroethyl) amine. Two cases, both afflicted with an extremely undifferentiated type of tumor, failed to respond to the initial course of treatment. The remaining cases had remissions of 2 to 6 months and have been followed for 2 years. A case of giant follicular lymphoma responded in a manner similar to those of lymphosarcoma.

Seven cases of chronic myeloid leukemia have responded to treatment with methyl-bis (β-chloroethyl) amine with transitory symptomatic and hematological improvement. We do not believe that the remissions induced were clinically significant.

Two cases of acute leukemia, 1 lymphatic and 1 myelogenous respectively have been treated without symptomatic relief, severe leukopenia developed in the latter case.

Seven cases of polycythemia rubra vera have responded favorably following courses of methyl-bis (β-chloroethyl) amine with remissions of from 3 to 17 months. Six continue in their initial remission. The hematological picture in each reverted to normal values within 1 month and remain within normal values. One case although free of symptoms had a moderate increase in the hemoglobin and erythrocyte levels and was treated 6 months after his first course.

The remissions produced in Hodgkin's disease and lymphosarcoma and in some cases of chronic lymphatic leukemia are sufficient to warrant extended study with a view to using methyl-bis (β-chloroethyl) amine or a related compound as an adjunct to roentgen therapy. The remissions produced in cases of polycythemia rubra vera are comparable to those following treatment with radioactive phosphorus and possibly superior to roentgen therapy. The nitrogen mustards have not been proven superior to roentgen therapy in the treatment of any of the lymphomas.

REFERENCES

1. Gilman, A., and Philips, F. S. The Biological Actions and Therapeutic Applications of the β-Chloroethyl Amines and Sulfides. Science, **103**:409-415. 1946.

   This article refers to the work done by A. Gilman, L. Goodman, C. E. Lindskog, and J. Dougherty at the New Haven Hospital.

2. Goodman, L. S., Wintrobe, M. M., Dameshek, W., Goodman, M. J., Gilman, A., and McLennan, M. T. Nitrogen Mustard Therapy. Use of Methyl-Bis(Beta-Chloroethyl)Amine Hydrochloride and Tris(Beta-Chloroethyl)Amine Hydrochloride for Hodgkin's Disease, Lymphosarcoma, Leukemia and Certain Allied and Miscellaneous Disorders. J.A.M.A., **132**:126-132. 1946.

3. Jacobson, L. O., Spurr, C. L., Barron, E. S. G., Smith, T., Lushbaugh, C., and Dick, G. F. Nitrogen Mustard Therapy. Studies on the Effect of Methyl-Bis (Beta-Chloroethyl) Amine Hydrochloride on Neoplastic Diseases and Allied Disorders of the Hemopoietic System. J.A.M.A., **132**: 263-271. 1946.

   These authors have another article with similar title in press for publication in the Journal of Clinical Investigation.

Abstracts

Reports of Research

Recent Advances in Cancer Research. LEWISOHN, R. [New York, N. Y.] *J. Mt. Sinai Hosp.*, **12**:464-497. 1945.

This article is confined to a discussion of the recent work in carcinogenic substances, as the aromatic hydrocarbons, with special reference to the conversion of physiological bile acids, and the relationship of sex hormones to carcinogenesis. The influence of female sex hormones on cancer formation is illustrated by its carcinogenic capacities when the hormone is injected in great quantity into experimental animals. This is regarded as of particular significance in the light of present treatment of the menopausal syndrome. Testosterone is discussed briefly as inhibitory to tumor formation. A report from the Cancer Research Laboratory of the Mt. Sinai Hospital is given. —A. C.

Nouvelles expériences sur l'action cancérigène chez la souris de l'insaponifiable du foie de sujets cancéreux. [New Experiments on the Carcinogenic Activity in Mice of the Non-Saponifiable Fraction of Liver from Cancer Patients.] SANNIE, G., TRUHAUT, R., and GUÉRIN, M., *Bull. Assoc. franç p. l'étude du cancer*, **32**:44-56. 1944.

Livers without metastases (group 1) from 5 patients and livers with metastases (group 2) from 5 patients, who had died from epidermoid or glandular carcinoma, were treated in the following manner. After desiccation, the liver pulp was treated by hot benzene and the lipid fraction obtained saponified by potassium hydroxide in alcohol. The non-saponifiable fraction was extracted by petroleum ether and dissolved in olive oil (0.20 gr. per cc.). The livers of 12 patients (group 3) who had died from non-neoplastic diseases, were used as controls.

One cc. of the extract solution from each group was injected subcutaneously in the dorsal region of mice, 20 mice being used for each group. No tumors were obtained with the non-saponifiable fraction from control livers. Four tumors (3 sarcomas and 1 mammary adenocarcinoma) developed in the mice injected with the fraction from group 1, and 2 tumors (1 sarcoma and 1 epidermoid carcinoma) in the mice injected with the fraction from group 2. These tumors appeared 16 months or more after the moment of inoculation.—C. A.

Action du méthylcholanthrène sur une cicatrice cutanée glabre, chez la Souris. [Action of Methylcholanthrene on a Cutaneous Scar Deprived of Hair, in Mice.] LACASSAGNE, A., and LATARJET, R. [Pasteur Inst., Paris, France] *Compt. rend. Soc. de biol.*, **139**:443-445. 1945.

Methylcholanthrene applied to a surgical cutaneous scar deprived of hair follicles, produced a cancer only at the periphery of the scar, in the proliferative zone of healing. No cancer developed in other parts of the cicatrix. When 1 application of a low concentration of methylcholanthrene was followed by a second of greater strength, fewer tumors developed than in the controls.—R. J.

Fréquence des ostéosarcomes à la suite d'injections intraveineuses de mésothorium chez le Lapin. [Production of Osteosarcomas in Rabbits after Intravenous Injection of Mesothorium.] GRICOUROFF, G. [Curie Foundation, Paris, France] *Compt. rend. Soc. de biol.*, **139**:558-560. 1945.

Intravenous injections of 20 to 50 micrograms of mesothorium into 12 rabbits were followed by the death of 5 animals before the tenth month. Among the 7 surviving rabbits, 5 developed osteosarcomas. Fracture sites were not always the place of localization of the cancerous growths. Very small amounts of radioactive substances were successful in producing tumors, but larger amounts apparently destroyed the susceptible cells since no growths appeared.—R. J.

Différences de réaction chez le Rat et chez le Cobaye, après injections intramusculaires de benzopyrène. [Difference in the Response to Intramuscular Injections of Benzopyrene in Rats and Guinea Pigs.] CHEVALLIER, A., DENOIX, P., and MANUEL, S. [National Inst. of Hygiene and Cancer Inst. Faculty of Med., Paris, France] *Compt. rend. Soc. de biol.*, **139**:598-599. 1945.

Benzpyrene produced tumors in 80% of the white rats treated. Guinea pigs were, on the other hand, almost completely refractory to the action of this chemical, even when large doses were used. The authors tried to establish whether the differences were due to a different metabolism of the product in the two animals. Spectrophotometric technics were used to detect the presence of the carcinogen and to localize and measure the amounts of the chemical in the host after certain lengths of time. The conclusions were that benzpyrene remains at the site of injection, and is eliminated very slowly in the case of the rat, an observation which was not seen in the guinea pigs. Here, the oil solution diffused widely and was relatively rapidly eliminated.—R. J.

Analyse du pouvoir cancérigène de différentes fractions d'une huile anthracénique. [Study of the Carcinogenic Action of Different Fractions of an Anthracenic Oil.] LACASSAGNE, A., RUDALI, G., and BUU-HOÏ, N.-P. [Polytechnic Sch., Paris, France] *Compt. rend. Soc. de biol.*, **139**:941-942. 1945.

Experimental study in mice (strain XVII) with an anthracenic oil showed that the acid fraction of the oil had the property of diminishing the carcinogenic action of the non-saponifiable fraction. The acid fraction, in some cases, was not responsible for the production of stomach cancer in mice.—R. J.

Tumours Produced by 2-Anthramine. BIELSCHOWSKY, F. [Univ. of Sheffield, Sheffield, England] *Brit. J. Exper. Path.*, **27**:54-66. 1946.

A solution of 2-anthramine in acetone was applied to the skin of 19 male rats (10 Wistar, 9 piebald). Between the 180th and 260th day spindle-cell tumors of the dermis

---

For information regarding microfilm copies of articles, abstracts of which appear in *Cancer Research*, application should be made to the Photoduplication Section, Army Medical Library, 7th Street and Independence Avenue, S.W., Washington 25, D. C.

appeared in 7, and basal-cell carcinomas in 3 of the Wistar rats at the site of application. Between the 224th and 293rd day similar spindle-cell tumors appeared in 2, and squamous and basal-cell carcinomas in 1 rat of the piebald series. The remote tumors (1 adenocarcinoma of the cecum, 2 epitheliomas of the ductus acusticus externus) were in piebald rats. No tumors of the liver were found in either strain. One of the spindled-cell tumors which was grafted showed no change in histological structure after 3 passages.—E. L. K.

Liver Necrosis and the Induction of Carbon Tetrachloride Hepatomas in Strain A Mice. ESCHENBRENNER, A. B., and MILLER, E. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **6**:325-341. 1946.

To investigate the relationship between liver necrosis and repair and the induction of hepatomas in A strain mice, a quantitative study of necrosis and repair following a single dose of carbon tetrachloride was made and the minimal necrotizing dose established. By using a graded series of necrotizing and non-necrotizing doses, a non-necrotizing dose was found which, when administered daily, would produce hepatomas, thus indicating that repeated liver necrosis and repair are, in all probability, not necessary for the induction of hepatomas. Also, although there was a correlation between the dose of carbon tetrachloride administered and both the degree of lobular disorganization and the increase of reticular fibers, some increase of reticular fibers was observed after the daily administration of sub-necrotizing doses of the agent. Some of the implications of these findings are discussed.—R. A. H.

L'action de l'hypophysectomie sur l'adéno-carcinome mammaire spontané de la Souris. [The Action of Hypophysectomy on the Evolution of Spontaneous Mammary Adeno-Carcinomas in Mice.] CHAMORRO, A., and DOBROVOLSKAÏA-ZAVADSKAÏA, N. [Inst. of Radium, Paris, France] *Compt. rend. Soc. de biol.*, **139**:614-617. 1945.

Hypophysectomy does not have any appreciable effect on the growth or the behavior of spontaneous adenocarcinomas in mice. The authors were unable to detect any change in the histological aspect of the cancer. Cancerous cells are not changed, whereas the normal mammary epithelium surrounding the tumor shows its usual process of atrophy following hypophysectomy.—R. J.

Sur l'origine musculaire locale de certaines métastases myocardiques dans le sarcome infectieux de la poule et son intérêt pour le mode d'action du virus. [The Local Muscle Tissue Origin of Certain Myocardial Metastases in Infectious Chicken Sarcoma and the Mode of Action of the Virus.] PEYRON, A. [Pasteur Inst. Paris, France] *Compt. rend. Soc. de biol.*, **135**:473-476. 1941.

A discussion is given of the author's theory concerning the myotropic nature and mode of action of the chicken tumor agent—*i.e.*, certain nodules of the myocardium, called "pseudo-metastases," are believed to be due to the proliferation of local muscle elements, caused by the polyvalent virus circulating in the blood.—G. H. H.

Quantitative Studies on the Latent Period of Tumors Induced With Subcutaneous Injections of the Agent of Chicken Tumor I. II. Estimation of the Latent Period. BRYAN, W. R. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **6**:373-377. 1946.

A method for a more accurate estimation of the latent period of Chicken Tumor I is given.—R. A. H.

Production expérimentale de tumeurs par piqures multiples sur la feuille de Chou cultivé. [Experimental Production of Tumors by Repeated Stitching on a Leaf of a Cultivated Cabbage.] ROSE, M. [Faculty of Sciences, Alger, France] *Compt. rend. Soc. de biol.*, **135**:1485-1487. 1941.

It is possible to imitate the appearance of small tumors by repeated pricking of a cabbage leaf in the same place. If the needle is soaked in bee venom, the effect on the leaf is the same, with increased intensity.—R. J.

Production experimentale de tumeurs sur la feuille de Chou par la technique du "puceron artificiel." [New Technic of Experimental Production of Tumors in the Cabbage Leaf. ROSE, M. [Faculty of Sciences, Alger, France] *Compt. rend. Soc. de biol.*, **135**:1491-1492. 1941.

The following technic was used: the opened end of a small ampoule of bee venom was gently introduced into the leaf tissue. After 5-6 days a tumor appeared at the site where the venom had been constantly applied.—R. J.

Tumeurs experimentales chez *Pelargonium zonale*. [Experimental Tumors in *Pelargonium Zonale*.] ROSE, M. [Faculty of Sciences, Alger, France] *Compt. rend. Soc. de biol.*, **135**:1493-1494. 1941.

The introduction of bee venom into flower staff of *Pelargonium zonale* produces the same effect as that obtained on the cabbage leaf. A localized tumor begins to appear 3-4 days after the beginning of the application of venom.—R. J.

Increase in Incidence of Lung Tumors in Strain A Mice Following Long-Continued Irradiation With Gamma Rays. LORENZ, E., HESTON, W. E., DERINGER, M. K., and ESCHENBRENNER, A. B. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **6**:349-353. 1946.

Strain A mice were exposed daily, over an 8 hour period, to 8.8 r of gamma radiation for 287 consecutive days and were autopsied when 10.5 to 11.5 months of age. Whereas 47.3% of the control animals had lung tumors at autopsy, 76.7% of the irradiated mice were so afflicted. The relationship of gamma radiation to lung tumors is discussed.—R. A. H.

Tumeurs expérimentales malignes et "splendothelan." [Experimental Malignant Tumors and "Splendothelan."] BESSEMANS, A., and VAN THIELEN, E. [Gland, Belgium] *Bull. Assoc. franç. p. l'étude du cancer*, **29**:182-187. 1941.

Prolonged daily feeding of large amounts of splendothelan, "a splenic and reticulo-endothelial product of Braunstein," (BRAUNSTEIN, A., *Zeitschr. f. Krebsforsch*, **39**:321. 1933.) to rats, mice, and guinea pigs did not produce any immunity to experimental tumors.—G. H. H.

Dosage d'acide ribonucléique dans des tissus cancéreux et sains. [Comparative Study of Amounts of Ribonucleic Acid Present in Cancerous and Healthy Tissues.] KHOUVINE Y., and GRÉGOIRE J. [Inst. of Physicochem. Biol., Paris, France] *Compt. rend. Soc. de biol.*, **139**:142-144. 1945.

Cells in process of active proliferation are 2 or 3 times richer in ribonucleic acid than resting cells of the same kind of tissue. Cancerous and healthy tissues of the uterus, stomach, breast, rectum, and also rat epithelioma and chick embryo were tested.—R. J.

Sur l'effet de l'aneurine (vitamine B^1) sur la croissance des tumeurs spontanées chez les Souris. [Action of Vitamin B_1 on the Growth of Spontaneous Tumors in Mice.] DOBROVOLSKAÏA-ZAVADSKAÏA, N. [Univ. of Paris, Paris, France] *Compt. rend. Soc. de biol.*, **139**:494-495. 1945.

A study of the action of vitamin B_1 on the growth of spontaneous breast cancers in mice showed that daily

subcutaneous injections of 0.04 to 0.06 mgm. and even 1 mgm. were well tolerated, and the rate of growth of the tumor was consistently slowed, the treated animals surviving longer than the controls. It is possible that the vitamin B_1 plays an active role in the metabolism of tumor glucides.—R. J.

L'Influence de l'α-tocophérol administré *per os* sur la croissance des tumeurs chez la Souris. [Influence of Feeding α-Tocopherol on the Growth of Tumors in Mice.] DOBROVOLSKAÏA-ZAVADSKAÏA, N., [Univ. of Paris, Paris, France] *Compt. rend. Soc. de biol.*, **139**:551-553. 1945.

Daily feedings of an oil solution of α-tocopherol, 50 parts per 100 and one drop per animal, delays the growth of spontaneous tumors in the breast of mice. However, similar amounts given once or twice a week seem to have a stimulating effect on the growths.—R. J.

L'effet de la nicotinamide (vitamine P.P.) sur la croissance des tumeurs chez les Souris. [Action of Vitamin PP on the Growth of Tumors in Mice.] DOBROVOLSKAÏA-ZAVADSKAÏA, N. [Univ. of Paris, Paris, France] *Compt. rend. Soc. de biol.*, **139**:592-594. 1945.

The subcutaneous injection of large amounts of nicotinamide (25 mgm. for each injection) lowered the rate of growth of breast tumors in mice and prolonged the life of the animals.—R. J.

Au sujet de l'influence du blé germé ajouté à la nourriture sur la croissance des tumeurs. [The Influence on the Growth of Tumors of Whole Wheat Added to the Food.] DOBROVOLSKAÏA-ZAVADSKAÏA, N. [Univ. of Paris, Paris, France] *Compt. rend. Soc. de biol.*, **139**:628-629. 1945.

Forty mice were fed different amounts of whole wheat. Among 57 adenocarcinomas observed, 28 showed a low rate of growth, as compared with the controls, but the other 29 tumors showed a faster growth rate during the first months; later the growth became slower. Actually, no very significant changes were observed. The animals fed with whole wheat survived longer than the controls. —R. J.

Action favorisante nette d'injections répétées d'acide ascorbique sur l'évolution macroscopique et microscopique de l'épithélioma métastasant de Guérine du Rat blanc. [Increase of the Metastatic Power of Guérin's Metastatic Epithelioma in White Rats after Repeated Injections of Ascorbic Acid.] DRIESSENS, J. [Inst. of Cancer Research, Lille, France] *Compt. rend. Soc. de biol.*, **139**:1014. 1945.

Injections of ascorbic acid were started the day after the transplantation of the tumor and were continued until the death of the animal, a total of 0.250 to 0.600 gm. being injected. The treated mice survived longer than the controls, and microscopic metastases in the lungs, adrenals, kidneys and liver were more frequent in the experimental animals than in the controls.—R. J.

Note on the Enzymatic Activity of Normal and Neoplastic Tissues of the Rat. GREENSTEIN, J. P., and LEUTHARDT, F. M., [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **6**:317. 1946.

The activity of 12 enzyme systems were tested in several normal and neoplastic tissues of the rat. The data show that the range of enzymic activity among normal tissues is considerably greater than among the three transplanted tumors studied.—R. A. H.

Sulfhydryl Groups in the Cathepsin of Rat Lymphosarcoma. MAVER, M. E., and THOMPSON, J. W. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **6**:355-362. 1946.

The effects of the so-called sulhydryl reagents upon the activity of purified cathepsin of the Murphy rat lymphosarcoma indicate that this enzyme system contains -SH groups which are essential for its activity. These groups are oxidized and the enzyme thus inactivated by iodine and sodium iodosobenzoate. *p*-Chloromercuribenzoic acid also reversibly inactivates the enzyme, and the quantity needed for complete inactivation correlates closely with the total quantity of -SH groups as determined by chemical titration. The -SH groups necessary for enzymic activity do not combine extensively with 3-amino-4-hydroxyphenyldichlorarsine hydrochloride or with iodacetamide. The cathepsin activity is activated by sodium cyanide. —R. A. H.

Inhibition and Stimulation of Tumor Homoiotransplants by Prior Injections of Lyophilized Tumor Tissue. SNELL, G. D., CLOUDMAN, A. M., FAILOR, E., and DOUGLASS, P. [Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.] *J. Nat. Cancer Inst.*, **6**:303-316. 1946.

Intraperitoneal or subcutaneous injections of lyophilized A strain mammary carcinoma 15091a were given mice of the C57 brown, C57 black and B alb C stocks over a period of 7 weeks, and, 8 to 12 days after the final injection, fresh tumor tissue was inoculated subcutaneously. In the majority of the treated animals the inoculated tumor grew progressively and killed the host, whereas in the majority of the untreated control animals the tumor either did not grow or regressed after a period of growth. The growth of C57 black sarcoma L946-AII transplanted to F_1 hybrid mice (Swiss × B alb C) was also stimulated, but to a lesser degree, by the prior injection of the recipient mice with lyophilized sarcoma tissue. On the other hand, lyophilized C57 black myeloid leukemia C1498 tissue administered to C57 leaden mice produced a distinct inhibition of growth of subsequent leukemic transplants. The relation of the genetic theory of tumor transplantation to tumor-immunity experiments is discussed. —R. A. H.

Les Lymphosarcomes du Rat. [Rat Lymphosarcoma.] ROUSSY, G., GUÉRIN, M., and GUÉRIN, P. [Cancer Inst., Paris, France] *Bull. Assoc. franç. p. l'étude du cancer*, **30**:17-28. 1942.

Twenty lymphosarcomas were found in 4,000 autopsies of rats. Almost all the tumors appeared in albino rats, males and females. These tumors must be considered as spontaneous in origin, although most of the rats had been inoculated with various types of tumors or treated with various carcinogenic substances. All the tumors developed in the ileocoecal region with the exception of 2 cases. Histologically they represented, in 18 cases, reticulo-lymphoblastic sarcomas; in 2 cases, lymphoid sarcomas. Metastases were very frequent. The tumor was successfully transplanted in 3 cases among 7 trials. One tumor was carried for 20 passages.—R. J.

Les hépatomégalies par réticulo-endotheliose chez la souris. [Hepatomegalia in Reticulo-Endotheliosis in the Mouse.] ROUSSY, G., GUÉRIN, P., and GUÉRIN, M. [Univ. of Paris, Paris, France] *Bull. Assoc. franç. p. l'étude du cancer*, **32**:34-43. 1944.

Lesions of the reticular system of the liver, varying from reticulo-granulomatosis to reticulum-cell sarcoma, were found in 22 mice in a total of 2,000 animals examined. Most of these animals had been used for experimental purposes and had received tar, benzpyrene or methylcholanthrene or had been grafted with transplantable tumors.—C. A.

Un cas de chlorome transplantable du rat. [Transplantable Chloroma in Rats.] ROUSSY, G., GUÉRIN, M., and GUÉRIN, P. [Cancer Inst., Paris, France] *Bull. Assoc. franç. p. l'étude du cancer*, **30**:29-42. 1942.

A spontaneous chloroleukemia in a rat was transplanted

into 350 rats. Thirty-nine chloromas appeared, 5 of them regressed spontaneously, while 6 series of transplants were successful. Metastases existed in one third of the cases, especially in the lymph nodes and the kidneys. The authors stress the existence of a secondary leukemia in some cases, following the primary growth of the tumor, by diffusion of tumor cells. This fact is considered to indicate that the leukemic cell is biologically a tumor cell. In almost all cases protoporphyrine was present in the tumors, but this substance could not be identified with the green pigment characteristic of these tumors. During the first passages x-rays as well as an unbalanced diet favored the growth of the grafts. However, later on these factors seemed to have little influence.—R. J.

Étude de deux lymphosarcomes transplantables chez le rat blanc. [Study of Two Transplantable Lymphosarcomas in White Rats.] ROUSSY, G., GUÉRIN, M., and GUÉRIN, P. [Cancer Inst., Paris, France] *Bull. Assoc. franç. p. l'étude du cancer,* **30**:146-155. 1942.

Two spontaneous lymphosarcomas (tumors T 173 and T 163), located in the ileocecal region of the rat served for this study. These had an almost identical histological structure with the exception of the amount of stroma which was greater in tumor T 173 than in tumor T 163. The tumors were transplanted and each followed until the 7th and 20th passage, respectively. Although almost identical, these tumors showed different biological behavior. The grafts were successful in 38.5% of the cases with tumor T 163 but only in 16% of the cases with tumor T 173. Metastases were also more frequent with tumor T 163 (45% instead of 16% with tumor T 173). X-ray irradiation did not have any effect on the growth of the grafts of tumor T 173, although this agent did inhibit effectively tumor T 163. The conclusions were that 2 tumors of the same histological appearance may have different behavior as far as their development and their reaction to x-ray irradiation is concerned.—R. J.

Réticulo-endothéliose transmissible chez la souris après inoculation de sang d'un malade atteint de réticulose. [Human Reticulosis Transmitted to the Mouse by Inoculation of Blood.] GUÉRIN, P., and GUÉRIN, M. [Anti Cancer Center of Hotel-Dieu, and Cancer Inst., Paris, France] *Bull. Assoc. franç. p. l'étude du cancer,* **31**:29-37. 1943.

The blood from a patient with hyperthermia, splenomegaly, adenopathies, and nodular skin lesions at the site of biopsy (the biopsy showing reticulo-granulomatosis) was injected into hens, rabbits, and mice. Only the mice gave interesting results. Ten males received the patient's blood intracerebrally, and 10 females ½ cc. subcutaneously. Among the surviving animals 2 series of symptoms were observed: 3 mice had signs of paraplegia, 8 mice had an abdominal syndrome characterized by an ascites, the enlargement of the liver and the spleen. There was no constancy in the enlargement of lymph nodes. Histologically the lesions are described as reticulo-endotheliosis, especially of the liver. It was noteworthy that, besides the inflammatory process, a proliferative process of neoplastic type with vascular invasion by the proliferative elements took place. The concomitant existence of both inflammatory and neoplastic lesions may be interpreted as a transition from inflammation to neoplasia. The neoplastic elements are more evident after several passages from mouse to mouse. A discussion follows on the etiology of the disease.—R. J.

Les greffes en série des sarcomas transplantables du Rat chez les animaux résistants. [Serial Grafts of Transplantable Rat Sarcomas in Resistant Animals.] ROUSSY, G., GUÉRIN, P., and GUÉRIN, M. [Univ. of Paris, Paris, France] *Compt. rend. Soc. de biol.,* **139**:230-231. 1945.

It is the opinion of the authors that the discordance in the results of reinoculation of the same tumors in rats primarily resistant to a first inoculation, is due to the fact that different kinds of tumors were tested. They found that Jensen's sarcoma resulted in the immunity of the host following one injection. On the other hand, other varieties of sarcomas had to be injected several times before a strong immunity was developed in the host.—R. J.

Les greffes en série d'épithéliomas transplantables du Rat chez les animaux résistants. [Serial Grafts of Transplantable Rat Epitheliomas in Resistant Animals.] ROUSSY, G., GUÉRIN, M., and GUÉRIN, P. [Univ. of Paris, Paris, France] *Compt. rend. Soc. de biol.,* **139**:231-233. 1945.

Immunity is difficult to obtain by serial injections of transplantable rat epitheliomas. There are differences in acquired immunity with different varieties of tumors; for example, uterine epitheliomas do not cause immunization even after a relatively large number of inoculations. One animal, however, did give a positive result after the fifth injection.—R. J.

Premiers résultats concernant la transplantation chez le Rat de tissu en voie de cancérisation. [Preliminary Results Concerning the Transplanting in Rats of a Tissue in the Process of Becoming Cancerous.] ROUSSY, G., GUÉRIN, M., and GUÉRIN, P. [Univ. of Paris, Paris, France] *Compt. rend. Soc. de biol.,* **137**:759-760. 1943.

This technic of transplantation was used in the study of the mechanism of action of chemical carcinogenesis, and also to study the behavior of tissue grafts in the process of becoming cancerous. Preliminary results seem to indicate that: 1) auto-transplants of such tissues have more chances to develop cancer than homo-grafts; 2) the development of a tumor does not depend on the general dissemination in the organism of the carcinogenic substance, but is due to its local action.—R. J.

Augmentation du pouvoir métastasique de l'épithélioma transplantable de Guérin du Rat blanc sous l'action de la novocaine, en injections intrapéritonéales et péritumorales. [Increase of the Metastatic Power of Guérin's Transplantable Epithelioma in White Rats after Intraperitoneal and Peritumoral Injections of Novocaine.] DRIESSENS, J. [Inst. of Cancer Research, Lille, France] *Compt. rend. Soc. de biol.,* **139**:1022-1024. 1945.

Both intraperitoneal and peritumoral injections of novocaine were capable of increasing considerably the number of microscopic metastases in various organs. Instead of 7% of the mice showing as was observed on the average in the controls, 50 to 60% of them in the treated group showed microscopic metastatic lesions.—R. J.

A Transplantable Rat Tumor. WATTS, R. M., and ADAIR, F. L. [Univ. of Chicago, Chicago, Ill.] *Am. J. Obst. & Gynec.,* **50**:11-28. 1945.

A round cell sarcoma which appeared in a rat treated over a period of time with estrogens has been studied in successive transfer generations.—A. K.

La prophylaxie du Cancer. Etude expérimentale. [The Prophylaxis of Cancer. An Experimental Study.] MAISIN, J. [Louvain, Belgium] *Bull. Assoc. franç. p. l'étude du cancer,* **29**:6-23. 1940.

It was found that cancer induced in rats and mice, by agents such as benzpyrene, methylcholanthrene, or *p*-dimethylaminoazobenzene, could be influenced by diet in two ways: (1) The induction of some cancers could be retarded by certain pure substances that are not growth-inhibiting, such as glycuronic acid, nicotinamide, and vitamin B_2, mixed with pyrophosphate. (2) In other

experiments, with animals on a deficient diet and given *p*-dimethylaminozoabenzene, and similar compounds, the induction of liver tumors was prevented by making up the dietary deficiency. In the conclusion, the author states that a specific growth-inhibiting substance found in animal tissues, particularly in brain and liver, and effective in the prophylaxis of skin cancer induced in mice and benzpyrene and methylcholanthrene, is ether-soluble and thermostable.—G. H. H.

Essais de traitement, par la radiothérapie de contact, des tumeurs chez la Souris. [An Assay of Treatment of Mice Tumors by Contact Radiotherapy. DOBROVOLSKAÏA-ZAVADSKAÏA, N., and ENNUYER, A. [International Research Foundation, Paris, France] *Compt. rend. Soc. de biol.*, **139**:152-154. 1945.

The technic is more of a short-distance radiotherapy (3 to 5 cm.) than a real contact radiotherapy. Twenty tumors were treated, and 17 animals survived much longer than the controls. In many cases there was no complete destruction of the tumors, and recurrences were observed.—R. J.

Diminution de la fréquence d'apparition de l'adéno-carcinome mammaire dans une ligneé de Souris sujette a cette tumeur. [Reduction in the Frequency of Mammary Gland Adenocarcinoma in a Strain of Mice Having High Tumor Incidence.] CHAMORRO, A. [Pasteur Inst., Paris, France] *Compt. rend. Soc. de biol.*, **139**:660-661. 1945.

Former investigations have shown that female white mice of the strain RIII (Dobrovolskaïa-Zavadskaïa) give a percentage of 53.5% of spontaneous mammary adenocarcinomas. Almost a 100% of the males of the same strain treated with estrogens developed mammary carcinomas (Lacassagne). These observations, in both females and males, were not substantiated by this study in which 6 generations of females, all suckled by mothers bearing carcinomas, and 10 males were treated with estrone benzoate. All animals were kept on a low fat, low protein diet.—R. J.

Sur une fraction, ayant des propriétés toxiques, isolée des tumeurs spontanées de la mamelle de souris. [A Fraction with Toxic Properties Isolated from Spontaneous Mammary Tumors in the Mouse.] DOBROVOLSKAÏA-ZAVADSKAÏA, N., and ZÉPHIROFF, P. [Univ. of Paris, Paris, France] *Compt. rend. Soc. de biol.*, **135**:602-605. 1941.

A substance with toxic properties, not salted out by sodium chloride but precipitated by absolute alcohol, was isolated from a mammary adenocarcinoma of the mouse. —G. H. H.

Contribution à l'étude pathogénique des cancers épithéliaux des parenchymes glandulaires. [A Study of the Pathogenesis of Carcinoma of the Parenchymal Glands.] DOUBROW, S. *Bull. Assoc. franç. p. l'étude du cancer*, **29**:164-181. 1941.

The pathogenesis of cancer may be regarded under two aspects: 1) the mechanisms by which any somatic cell becomes a cancerous cell, and 2) the factors that release these mechanisms. The author discusses the problem in relation to cancer of the breast and of the gastric mucosa. After histophysiological studies of surgical specimens, he concludes that an analogy exists between the phenomenon of carcinogenesis of the secretory adenomatous cell of the mammary gland and that of the heterotopic secretory cell of the gastric mucosa. Both types of cell seem to have lost their permeability for mineral substances, and retain organic products of nuclear metabolism. A theory of carcinogenesis is outlined. —G. H. H.

Clinical and Pathological Reports

Clinical investigations are sometimes included under Reports of Research

MULTIPLE TUMORS

Les tumeurs doubles à tissus histologiquement différents. [Double Tumors with Different Histological Structures] DELCOURT, R. [Schaerbeek Hosp., Brussels, Belgium] *Bull. Assoc. franç. p. l'étude du cancer*, **31**:129-141. 1943.

Double cancers with different histological structure are relatively rare (1.56%). They can grow simultaneously in the same patient or be successive. The possible clinical latency of one of the tumors may be misleading in the appreciation of their real chronological appearance. The evolution of such tumors seems more rapid than other cancers. Their study does not throw any light on the etiological problem of cancer in general.—R. J.

DIAGNOSIS—GENERAL

Critical Remarks Concerning the Nature of the Polarographic Serum Reactions for Cancer. BRDIČKA, R. [Inst. of Radio-Therapy, Bulovka Hosp., Prague, Czechoslovakia] *Acta radiol. et cancerol. Bohemiae et Moraviae*, **2**:7-16. 1939.

The theoretical foundation of two kinds of polarographic serum reactions concerning typical changes in human serum from individuals suffering from cancer, sarcoma, inflammations, infections, or certain bile or liver disorders is discussed in detail.

Some experimental facts elucidating the nature of the pathological changes in sera are reviewed. The erroneous objections to the author's original interpretation, brought forward by certain authors, especially by V. Moravek and his collaborators, are dealt with. It is shown that the experimental results of the latter authors concerning the polarographic examination of two horse sera, one normal and the other sarcomatous, are not, as these authors believe, contradictory to the present author's theory, but on the contrary, when investigated more exhaustively than these authors were able to do, even yield valuable evidence in support of it. Closely analogous results to those of the authors mentioned have been obtained by the present author with human sera and are fully explained.

Finally the cause of the pathological change in the blood stream is discussed.—Author's summary.

Critical Examination of the Polarographic Test for Cancer in Deproteinated Sera. BRDIČKA, R. NOVÁK, F. V., and KLUMPAR, J. [Inst. of Radio-Therapy, Bulovka Hosp., Prague, Czechoslovakia] *Acta radiol. et cancerol. Bohemiae et Moraviae*, **2**:27-41. 1939.

The aim of this communication is a critical examination of the polarographic test for cancer given by serum filtrates after deproteination with sulfosalicylic acid. On the basis of experiments with different deproteinating agents, evidence of the albumose-like character of the substance, the increase of which indicates the pathological state, is brought forward.

A detailed study of the deproteination of sera with sulfosalicylic acid is described which reveals some important factors in diagnostic tests: (1) proper caution must be paid to the rigorous maintenance of a fixed time interval between the precipitation of proteins and the filtration of the precipitate, (2) the test is improved by introducing an excess of ammonia in buffered cobaltihexamine solution. The improved test was checked by tests on 183 cancer patients and was found positive in 177 cases.

A modified mode of procedure was worked out in which 0.1 cc. of blood taken from the finger was polarographically examined instead of serum. The diagnostic results are illustrated by concrete examples.

The former hypothesis that the observed increase of the protein degradation products in the blood is to be ascribed to the dispersion of foreign proteins in the bloodstream is supported by experiments on the parenteral application of milk to normal individuals. After the application of milk a positive filtrate test is obtained, which after a certain time again returns to normal.—Author's summary.

Test Case to Show Value of Cervical Cytology Smear in Uterine Cancer Diagnosis. AYRE, J. E., BAULD, W. A. G., and KEARNS, P. J. [McGill University, Montreal, Canada] *Am. J. Obst. Gynec.*, **50**:102-105. 1945.

Early cervical cancer has been detected by the cervical smear method when the lesion has not been palpable, nor diagnosed by 50% of the surgical biopsies.—A. K.

THERAPY—GENERAL

Les résultats de la radiothérapie des cancers cervico-utérins du premier degré. [Results of the Radiotherapy of Cancers of the Cervix Uteri at an Early Stage.] LABORDE, S. [Cancer Inst., Paris, France] *Bull. Assoc. franç. p. l'étude du cancer,* **30**:43-51. 1942.

One thousand eight hundred cervical epitheliomas were examined at the Cancer Institute from 1922 to 1937; only 65 of these were observed early in their development. Fifty-seven patients were treated at an early stage by irradiation. Among these, 66.6% were considered cured after a period of observation averaging 15 years, although it is known that recurrence of the disease may be observed after 20 years. The late appearance of metastases remains unexplained. The recurrence of the tumor in its original location is relatively rare, distant metastases being more frequent. Local bacterial infection makes the treatment sometimes difficult. A discussion is given concerning the degree of radio resistance of the different anatomical types of cancer. The age of the patient does not seem to play a predominant rôle in the degree of malignancy of these tumors.—R. J.

Giant Hemangio-Endothelioma with Thrombocytopenic Purpura. Results of Roentgen Therapy. RHODES, A. W., and BORRELLI, F. J. [Flower and Fifth Ave. Hosps., New York, N. Y.] *Am. J. Roentgenol.*, **52**:323-326. 1944.

A giant hemangio-endothelioma of the face, shoulder, and thorax of an infant regressed under heavy doses of deep x-ray therapy, and an associated thrombocytopenic purpura disappeared.—M. H. P.

The Rôle of Castration as Auxiliary Treatment in Carcinoma of the Breast. Report of a Case. WOLF, B. S. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **12**:907-916. 1945.

The use of castration as a palliative measure and auxiliary treatment in carcinoma of the breast is discussed and its best indications are the presence of active tumor of the breast or when evidence of ovarian activity is present. Spontaneous menopause does not exclude the possibility of satisfactory palliation. In males with carcinoma of the breast and active disease, orchidectomy is indicated. Androgens and estrogens are contraindicated in both sexes.—A. C.

Results of Various Types of Treatment in Adenocarcinoma of the Endometrium. MCLENNAN, C. E. [Univ. of Utah, Salt Lake City, Utah] *Am. J. Obst. & Gynec.*, **50**:254-262. 1945.

Results of treatment of 225 patients with adenocarcinoma of the uterine corpus have been reviewed. The absolute 5 year cure rate has been 45%. Poor results follow routine use of radium and x-ray alone. On the other hand, certain patients so treated may be salvaged many years later by hysterectomy for either recurrent or persistent carcinoma. Good results have followed the use of total hysterectomy, with or without pre- or postoperative irradiation. It is felt that preoperative irradiation will add nothing to the ultimate cure rates. The final results in the treatment of carcinoma of the uterine corpus are predetermined to a considerable extent by the presence of the metastases, the type and extent of medical and surgical complications, age, weight, and nutritional status of the patient.—A. K.

Le traitement des papillomes vulvaires par l'application locale de colchicine. [The Treatment of Papillomas of the Vulva by the Local Application of Colchicine.] BOURG, R., and DUSTIN, P., JR. [Hosp. of Univ. St.-Pierre, Brussels, Belgium] *Presse méd.*, **53**:578-579. 1945.

A case report. Most of the tumor disappeared in one month following the daily application of colchicine (0.05 gm. in lanolin).—C. A.

Radiation Therapy in Uterine Fibroids. PEAKE, J. D. [Mobile, Ala.] *South. M. J.*, **38**:480-484. 1945.

Two hundred and ninety cases, of a total of 300 uterine myomas with bleeding, were treated by radiation. One hundred and eighty-six received roentgen therapy, 86 had radium (dilatation and curettage), and 18 had combined x-ray and radium therapy. The uterine hemorrhage was stopped in all but two cases. In one of these cases there was a uterine fibroid with a tubo-ovarian abscess found at operation, and in the other a pedunculated submucal fibroid was observed. Whereas 3 patients had continued pain after uterine bleeding ceased, they showed little improvement following surgery. No explanation for the pain was found.—W. A. B.

Radio-Sensitive Parasellar Tumors. LAWRENCE, W. S., and ROBINSON, W. W. [Memphis, Tenn.] *South M. J.*, **38**:510-513. 1945.

Extra-sellar tumors may simulate true pituitary tumors, although no sign of enlargement of the sella is found on x-ray examination. These tumors are (1) craniopharyngiomas, or tumors of Rathke's Pouch, (2) meningiomas of the tuberculum and diaphragm of the sella, (3) chiasmal gliomas, and (4) aneurysms of the intracranial portion of the internal carotid artery. Radiation has no effect on the meningiomas, and with craniopharyngiomas and gliomas, the effect of radiation is poor and only temporary. True adenomas of the pituitary show marked sensitivity to radiation. A case is reported in which a parasellar tumor showed striking response to radiation, suggesting that it was of true pituitary cell origin. The patient's symptoms were similar to those seen with chromophobe adenoma.—W. A. B.

Supervoltage Radiation. A Review of the Cases Treated During an Eight Year Period (1937-1944 Inclusive). HOLMES, G. W., and SCHULZ, M. D. [Massachusetts General Hosp., Boston, Mass.] *Am. J. Roentgenol.*, **55**:533-554. 1946.

During 8 years (1937-1944) 1835 tumor cases have been treated by the authors with 1200 kv. x-rays. Most of these were malignant; the results are analyzed as separate groups and compared with published results of 200 kv. or other therapy. In general it can be stated that the most important advantage of supervoltage is the fact that skin damage can be avoided. The total dose which can be administered depends upon the reaction of the deep-seated organs in the paths of the rays. Deep seated, localized lesions are most favorable for treatment, and the life of patients with these can be prolonged and made more comfortable. More information is necessary before

final conclusions can be drawn; in the meantime the authors recommend supervoltage therapy for selected cases of carcinoma of the cervix, bladder, lung, large intestine, rectum, anus, and tonsil, embryoma of the testicle, localized lymphoma, and Ewing's tumor.—E. H. Q.

Radiation

Ulcerative Colitis after X-Ray Treatment for Cancer of Uteris. Engelbreth-Holm, J. [Radiumstation and Univ. Inst. of Path. Anat., Copenhagen, Denmark] *Acta med. Scandinav.,* **116**:308-321. 1944.

Five case reports are described. In 4, the patients developed acute, fulminant ulcerative colitis, which had its onset during or several days after x-ray therapy for cancer of the cervix and which resulted in death within a few weeks. In the fifth case, the patient developed the same complication 6 weeks after irradiation of the abdominal lymph nodes for Hodgkin's disease and died in 2 weeks. The ulcerative colitis is attributed to at least two factors: (1) an increased permeability of the intestinal epithelium to bacteria, brought about by the irradiation, and (2) a tendency to spasms, resulting from the irradiation and also from the subsequent inflammation.—G. H. H.

The Effect of Irradiation on the Function of the Ovary in Young Girls. Kaplan, I. I. [Bellevue Hosp., New York, N. Y.] *Am. J. Obst. & Gynec.,* **50**:340-341. 1945.

A 4½ year old girl with neurogenic tumor in the pelvic region received intensive irradiation. She menstruated for the first time at 12 years of age and regularly thereafter until 19, at which time she died as a result of recurrence of the neoplasm.—A. K.

Skin and Subcutaneous Tissues

Sweat Gland Tumors of the Vulva, Benign (Hidradenoma) and Malignant (Adenocarcinoma). Novak, E., and Stevenson, R. R. [Johns Hopkins Med. Sch., Baltimore, Md.] *Am. J. Obst. & Gynec.,* **50**:641-659. 1945.

Sweat glands of the vulva give rise to a relatively rare but very interesting group of tumors. Clinically these growths are small and benign-looking, but the microscopic picture suggests malignancy and frequently the diagnosis of adenocarcinoma is made if the pathologist is not familiar with this type of lesion. There is only one case in the literature where unimpeachable evidence of malignant change has been demonstrated. In the series of 15 cases reported here, all tumors have been benign, and curable by simple excision. It is possible that rarely an adenocarcinoma of the vulva is of sweat gland origin, but such histogenesis is difficult to establish.—A. K.

Nervous System

Craniopharyngeoma and Suprasellar Adamantinoma. Globus, J. H., and Gang, K. M. [New York, N. Y.] *J. Mt. Sinai Hosp.,* **12**:220-276. 1945.

A review of 14 cases of hypophyseal duct tumor, grouped as cystic or solid, is made. The tumors were found to be derived from residues of the so-called craniopharyngeal (cranio-oral) duct. The tumors often implicated the floor of the third ventricle or broke through it to expand into the third ventricle, thereby causing hypothalamic disorders. Clinically, the cases presented symptoms and signs which offered diagnostic leads and could be divided into three groups: (a) vegetative disturbances, (b) general manifestations of increased intracranial tension, and (c) signs due to direct pressure by tumor. In addition x-rays disclosed calcifications in or about the sella turcica. Surgical intervention was found to give only temporary relief.—A. C.

Encephalo-Trigeminal Angiomatosis. Green, J. R. [Chicago, Ill.] *J. Neuropath. & Exper. Neurol.,* **4**:27-42. 1945.

Angiomatosis of the scalp, meninges and brain should be suspected when a child who has a "port-wine mark" in the area of the trigeminal nerve develops convulsions, hemiparesis, or mental retardation. The diagnosis is confirmed by the finding of sinuous, double-contoured calcifications in the roentgenograms of the skull. Localization of the pathological process may be aided by electroencephalography and pneumoencephalography and, in the author's opinion, should be confirmed by stereoscopic angiography, especially in cases where there is no gross calcification. In the case reported, one month after extensive resection, the patient showed marked improvement but the author feels that final judgment concerning the results of surgical intervention should be suspended for a number of months or years.—A. C.

Blood Vessel Hyperplasia Masking Glioblastoma Multiforme. Report of a Case. Hawn, C. Van Z., and Ingraham, F. D. [Boston, Mass.] *J. Neuropath. & Exper. Neurol.,* **4**:364-369. 1945.

A case is presented which was diagnosed as a slow-growing fibrosarcoma after superficial biopsy of the cortex in the parieto-occipital area. The diagnosis was based on the presence of interlacing bundles of collagenous fibers due to the marked hyperplasia of the walls and adventitia of blood vessels in close proximity. Two days after biopsy the mass was removed and revealed, on section, the typical picture of glioblastoma multiforme underlying the area of original biopsy. Since many neurosurgical procedures are based on primary biopsy studies, such a potential source of error must be kept in mind.—A. C.

The Syndrome of the Posterior Inferior Cerebellar Artery Resulting from a Metastatic Neoplasm. Davison, C., and Spiegel, L. A. [New York, N. Y.] *J. Neuropath. & Exper. Neurol.,* **4**:172-177. 1945.

Two cases are presented showing the syndrome of occlusion of the posterior inferior cerebellar artery due to metastatic neoplasm of the latter part of the medulla. In both cases the primary site was in the lungs. In one of the cases, while the clinical picture was not too clear-cut, the necropsy findings bore out the diagnosis. In the second case, the signs and symptoms were typical, but necropsy was not performed for verification.—A. C.

Hemangioma of the Medulla Oblongata. Owen, C. I., Webster, J. E., and Gurdjian, E. S. [Detroit, Mich.] *J. Neuropath. & Exper. Neurol.,* **4**:291-294. 1945.

A case of hemangioma of the medulla oblongata is reported in which the clinical picture consisted of persistent dysphagia and left-sided cranial nerve signs. The onset was sudden and the termination rapid. On necropsy a cavernous hemangioma, which was considered the basis for the symptoms, was found in the floor of the fourth ventricle with hemorrhage into the tumor. Blood vessel tumors of the bulb are rare, especially when unassociated with similar lesions elsewhere in the body or nervous system.—A. C.

Breast

La cancérisation des mastoses. Maladie de Reclus, Mammite scléro-kystique. [The Cancerisation from Mastitis. Reclus Disease, Sclero-Cystic Mammitis.] Guérin, P., and Guérin, M., *Bull. Assoc. franç. p. l'étude du cancer,* **32**:2-19. 1944.

The incidence of malignant changes in chronic mastitis is over 10%. These changes generally occur in cases showing intense epithelial hyperplasia, particularly of the intracystic papillomatous type. Simple sclero-cystic forms very rarely give rise to cancer. A discussion with 15 case reports.—C. A.

Female Genital Tract

Leiomyoma of the Ovary Complicating Pregnancy. Moore, J. H. [Grand Forks, N. D.] *Am. J. Obst. & Gynec.,* **50**:224-226. 1945.

A case of leiomyoma of the ovary complicating pregnancy is reported. There are only two other reports where this type of neoplasm has been found in association with pregnancy.—A. K.

Dermoid Cyst of Ovary Containing a Structure Similar to a Human Body (Homunculus). Plaut, A. [Beth Israel Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.,* **12**:567-578. 1945.

A dermoid cyst is described containing a miniature human-like structure (homunculus) free in the lumen, with remnants of an umbilical cord. Pertinent literature and theories are discussed with emphasis on the organizer theory.—A. C.

Theca Cell Tumor of the Ovary in a 72 Year Old Woman. Wimpheimer, S. [New York, N. Y.] *J. Mt. Sinai Hosp.,* **12**:768-772. 1945.

Theca cell tumors in women long past menopause have been found to have a strong hormonal effect resulting in hyperplasia of the endometrium and development of feminizing characteristics such as atypical uterine bleeding and breast enlargement. In the case reported, a 72 year old woman, these changes took place 21 years after menopause.—A. C.

Primary Carcinoma of the Fallopian Tube. Ayre, J. E., Bauld, W. A. G., and Kearns, P. J. [McGill Univ., Montreal, Canada] *Am. J. Obst. & Gynec.,* **50**:196-202. 1945.

Case report.—A. K.

Primary Carcinoma of the Fallopian Tube. Mitchell, R. M., and Mohler, R. W. [Pennsylvania Hosp., Philadelphia, Pa.] *Am. J. Obst. & Gynec.,* **50**:283-292. 1945.

Case report and review of the literature.—A. K.

Carcinoma Corporis Uteri in 2 Sisters Aged 34 and 32 Years. Purdie, A. W. [North Middlesex County Hosp., London, England] *J. Obst. & Gynaec. Brit. Emp.,* **52**:575-579. 1946.

The statement that carcinoma corporis uteri never occurs before the menopause is erroneous. These tumors occurred in two sisters, married, age 32 and 34. A right ovarian cyst weighing 24 lbs. had been removed from the elder sister 10 years before. The breasts did not show any hyperplastic changes. Another sister had undergone hysterectomy for "a benign condition," details of which are not given. The mother died of cancer of the rectum at the age of 68. The daughters were not breast fed. The literature relating to early and familial cases of this form of cancer is reviewed.—E. L. K.

Quelques remarques sur le traitement radiothérapique des adénocarcinomes du col utérin. [Remarks on the Radiotherapy of Adenocarcinomas of the Cervix Uteri.] Baclesse, F., and Fernandez-Colmeiro, J. M. [Curie Foundation, Paris, France] *Bull. Assoc. franç. p. l'étude du cancer,* **30**:118-128. 1942.

The authors add the study of 56 cases of adenocarcinomas treated by radium therapy to the 364 other cases collected in world literature and draw the conclusion that radiotherapy alone gave an apparent 5 year cure in 101 cases (24%). Hence the discredit given to radiotherapy in the treatment of adenocarcinomas of the cervix uteri is not justified. Nevertheless, the combination of radiotherapy with surgery seems to give the most favorable results.—R. J.

Metastatic Adenocarcinoma of the Cervix Uteri Associated with Primary Gastric Cancer. Williams, E. L. [Vanderbilt Univ. Sch. of Med., Nashville Tenn.] *Am. J. Obst. & Gynec.,* **50**:342-344. 1945.

Cervical cancer representing a metastatic lesion from a gastric tumor is reported. An analogy to the Krukenberg tumor of the ovary is made, in that in both cases a growth of the gastrointestinal tract finds a suitable medium for proliferation in the genital tract.—A. K.

Early Cervical Carcinoma in Three Clinically Unsuspected Cases Incidental to Plastic Operations. Rubin, I. C. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.,* **12**:607-615. 1945.

During routine examination of tissue following a trachelorrhaphy in one case and the Fothergill operation in two other cases, early cervical carcinoma was discovered. In one, no evidence of recurrence has been determined after 7 years in spite of refusal of all further treatment. In another there has been no recurrence after more than 5 years following a course of radiation. In the third case, carcinoma has recurred in the cervical stump and uterine body in spite of ample x-ray and radium therapy.—A. C.

Carcinoma of the Cervix Complicated by Pregnancy. Wilson, J. R. [Univ. of Chicago, and Chicago Lying-in Hosp., Chicago, Ill.] *Am. J. Obst. & Gynec.,* **50**:275-283. 1945.

This is a review of the treatment of six cases. Carcinoma of the cervix is unusual during pregnancy, and when it is present, irradiation is the treatment of choice. A high fetal mortality is inevitable in these patients since it is usually unwise to resort to methods of treatment which attempt to preserve the fetus but which may decrease the chances for "cure" of the neoplasm.—A. K.

Le cancer du col restant et de la cicatrice vaginale après hystérectomie. Statistiques et deductions. [Cancer of the Cervical Stump and of the Vaginal Scar Following Hysterectomy. Statistics and Deductions.] Ponthus, P., and Rocher, P. [Anti-Cancer Center, Lyon, France] *Bull. Assoc. franç. p. l'étude du cancer,* **32**:26-32. 1944.

Seven cases of cancer of the vaginal scar following total hysterectomy and 41 cases of cancer of the cervical stump following subtotal hysterectomy are reported. In the latter group, cancer appeared within a year in 15 cases; in 6 of these the tumor was a secondary growth of utero-ovarian origin and in the 9 others the tumor was primary. It is the authors' opinion that in these cases a tumor existed at the time of the operation, but passed unnoticed. Cancer appeared after a period of 3 years in 26 cases. These growths are considered as true tumors of the cervical stump. The cervical stump is more apt to become cancerous than the ordinary cervix.—C. A.

Male Genital Tract

The Management of Malignant Tumors of the Testis. Pendergrass, E. P., Chamberlin, G. W., Selman, J., and Horn, Jr., R. C. [Hosp. of Univ. of Penn., Philadelphia, Pa.] *Am. J. Roentgenol.,* **55**:555-574. 1946.

In a series of 43 patients with known malignant tumors of the testis, the over-all 5 year survival rate was 42%. Of patients free from demonstrable metastases at admission, treated by orchiectomy and x-ray therapy, 71% survived 5 years; of those having metastases, only 30%. Although it is usually stated that a seminoma offers a better prognosis than a teratoma, in this series the 2 showed no significant difference. There was no significant difference between groups irradiated preoperatively and those irradiated postoperatively, but, in view of the

malignancy of the disease, it is recommended that such treatment be preoperative or immediately postoperative. The authors' scheme of irradiation is given in detail. —E. H. Q.

Urinary System

Hypernephroid Carcinoma of the Kidney or Adrenal (?) and Pregnancy. Lash, A. F. [Michael Reese Hospital, Chicago, Ill.] *Am. J. Obst. & Gynec.*, **50**:221-223. 1945.

Case report.—A. K.

Intrathoracic Tumors

Un cas de pachypleurite cancéreuse à évolution rapide. [A Case of Cancerous Pachypleuritis of Rapid Evolution.] Delcourt, R., and Tenret, J. [Sanatorium Les Pins, La Hulpe, and Hosp. of Schaerbeek, Schaerbeek, Belgium] *Bull. Assoc. franç. p. l'étude du cancer*, **30**:156-167. 1942.

In this 54-year old patient, the start of the tumor signs coincided with local trauma to the chest. The development of the lesion was very rapid. The origin of the tumor is discussed. Many arguments support the opinion that the tumor, although resembling a primary tumor of the pleura, was in reality of bronchial origin. A survey of the abundant literature on the subject showed that actually in only one case was the diagnosis of primary pleural tumor certain.—R. J.

Roentgenologic Appearance and Pathology of Intrapulmonary Lymphatic Spread of Metastatic Cancer. Mueller, H. P., and Sniffen, R. C. [Mass. General Hosp., Boston, Mass.] *Am. J. Roentgenol.*, **53**:109-123. 1945.

Ten cases of lymphangitic carcinosis of the lungs, seen in recent years at the Massachusetts General Hospital, are presented to illustrate the lymphatic spread of cancer. Autopsy was performed on 7 of the group. The roentgenologic appearance presents a typical pattern which is entirely different from that of the usual pulmonary metastasis. It is characterized by a network of increased density, radiating from enlarged hilar lymph nodes and trabeculating through the lung fields in an uneven manner, and is interspersed by numerous fine nodules. It is more marked in the lower and central portions of the lungs and occasionally may be unilateral. Differential diagnoses of miliary tuberculosis, pulmonary edema and congestion, sarcoid, pneumoconiosis, and bronchiectasis, among others, must be ruled out. The microscopic picture in the lungs is characterized by widespread permeation of the lymphatics by tumor cells and, in a certain number of instances, profound arterial damage. Anaplasia of the tumor, rather than its primary site, appears to be the important factor in this form of dissemination.

The age and sex incidence, the mode of spread and the arterial changes are discussed. The common clinical findings are dyspnea, cyanosis, productive cough and rapid cachexia. Pulmonary signs are minimal, but pulmonary symptoms overshadow those which arise from the primary tumor.—E. H. Q.

Leiomyosarcome de la paroi thoraco-abdominale. [Leiomyosarcoma of the Thoraco-Abdominal Wall.] Guérin, P., Guérin, M., and Tailhefer, A. [Anticancer Center of Hôtel-Dieu, and Cancer Inst., Paris, France] *Bull. Assoc. franç. p. l'étude du cancer*, **31**:47-54. 1943.

A case is described in which there was evidence of malignancy although no metastases occurred. The vascular origin of the tumor was proved histologically.—R. J.

Gastrointestinal Tract

Leiomyoma of the Stomach. Meissner, W. A. [Mass. Dept. of Public Health, Boston, Mass.] *Arch. Path.*, **38**:207-209. 1944.

The growth was observed in 46% of a series of 50 routine autopsies on adults. The tumors arise most frequently in the fundus near the cardia. They are usually without clinical significance though they may sometimes enlarge and cause ulceration of the mucosa.—J. G. K.

Fecal Impaction Complicated by Undetected Malignancy and Coma. Report of a Case. Landsman, A. A. [North Hudson Hosp., Weehawken, N. J.] *M. Rec.*, **159**:37. 1946.

Case reported in which fecal impaction with resulting intestinal obstruction and coma of undetermined etiology was relieved by multiple high irrigations. Six weeks later a similar episode occurred; operation, refused on the first admission, revealed concomitant adenocarcinoma of the sigmoid. The importance of sigmoidoscopy after treatment of fecal impaction is stressed.—V. J. L.

Note sur 110 cas de cancer du rectum. [Note on 110 Cases of Cancer of the Rectum.] Ducuing, J., and Grimoud, M. [Anticancer Center, Toulouse, France] *Bull. Assoc. franç. p. l'étude du cancer*, **30**:138-145. 1942.

Most of the cases were treated by transsacral amputation of the rectum. By using this technic, more advanced cases can be operated on than by using the abdominal technic. A discussion follows concerning cancers developed on preexistent polyposis of the rectum, cases where the anal sphincter may be conserved, post-operative mortality and morbidity. The metastases are relatively rare. The liver metastases, after operation, frequently follows the recurrence of the disease *in situ*. The rôle of the medial hemorrhoidal veins in the propagation of the disease is stressed.—R. J.

Peritoneum and Retroperitoneum

Pseudomyxoma Peritonaei. Report of a Case with Unusual Roentgen Findings. Weig, C. G., Koenig, E. C., and Culver, G. J. [Buffalo General Hosp., Buffalo, N. Y.] *Am. J. Roentgenol.*, **52**:505-509. 1944.

A report of a case exhibiting the usual features of pseudomyxoma of the peritoneum, and the unusual feature of calcification of secondary peritoneal implants is given. The patient at the time of writing was alive and well 13 years after the onset of symptoms; some of the nodules had been removed surgically and others showed increasing calcification during the last 8 years.—M. H. P.

Bone and Bone Marrow

A Case of Myelomatosis with Diffuse Plasma Cell Infiltration of Lymph Nodes, Liver, Spleen, Kidneys, and Lungs. Hansen, A. T. [Praestø District Hosp., Naestved, Denmark] *Acta med. Scandinav.*, **115**:514-523. 1943.

A case of a 62 year old man is described in detail. The relation between myelomatosis, plasma cell leukemia, and other malignant diseases of the myelopoietic system is discussed. Plasma cell leukemia is here regarded as a leukemic form of myelomatosis.—M. H. P.

Les diversités anatomo-cliniques et évolutives des tumeurs à myéloplaxes des os longs. [Difference in Anatomical and Clinical Forms and Difference in the Evolution of Myeloplaque Tumors of the Long Bones.] Delarue, J., and Denoix, P. [Cancer Inst., Paris, France] *Bull. Assoc. franç. p. l'étude du cancer*, **31**:116-127. 1943.

The benign nature of these tumors is now well established. Nevertheless there exist important differences in their evolution and their response to surgical treatment or radiotherapy. The authors, after study of the literature available at the Cancer Inst. of Paris, believe that a difference in the histological form and anatomical behavior of such tumors is responsible for the difference in therapeutic results. They believe that an optimal time for the surgical intervention should be selected, depending on the "reactional state" of the tumor.—R. J.

Eosinophil Granuloma of Bone—Schüler-Christian's Disease. ENGELBRETH-HOLM, J., TEILUM, G., and CHRISTENSEN, E. [Univ. Inst. of Path. Anat., Radium Station, and Rigshosp., Copenhagen, Denmark] *Acta med. Scandinav.*, **118**:292-312. 1944.

Five cases are reported of the so-called eosinophile granuloma of bone, in children 2 to 9 years old, presenting clinically and morphologically a gradual transition to the fully developed Schüller-Christian's syndrome. Eosinophile granuloma is considered a monosymptomatic form of Schüller-Christian's disease that often heals without becoming generalized.—G. H. H.

Neurofibromatosis of Bone. FRIEDMAN, M. M. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] *Am. J. Roentgenol.*, **51**:623-630. 1944.

Four cases of neurofibromatosis with bone involvement are presented, in one of which neurofibromatous tissue was demonstrated in the marrow cavity of the femur. Roentgenograms, a photograph, a photomicrograph, and a literature review with bibliography accompany this report.—M. H. P.

Osteoid-Osteoma. A Review of Portions of the Literature and Presentation of Cases. LEWIS, R. W. [Hosp. for Special Surg., New York, N. Y.] *Am. J. Roentgenol.*, **52**:70-79. 1944.

Nine proved and 2 probable cases of osteoid-osteoma are presented which conform clinically, roentgenologically, and pathologically to the criteria of Jaffe and Lichtenstein (*Arch. Surg.*, **31**: 709. 1935; *J. Bone & Joint Surg.*, **22**: 645. 1940) for diagnosis of this disease. In 1 case, the tumor occurred in an innominate bone, a site apparently not previously reported. Simple removal of the osteoid-osteoma (the nidus) brought prompt cure in all cases in which it was performed. Failure to remove the nidus resulted in persistence of symptoms.—M. H. P.

Osteoid-Osteoma of the Head of the Radius. Case Report. STAUFFER, H. M. [Temple Univ. Med. Sch. and Hosp., Philadelphia, Pa.] *Am. J. Roentgenol.*, **52**:200-202. 1944.

In the case reported, the original pathological diagnosis was "healing benign giant-cell tumor." However, a review of the roentgenograms suggested osteoid-osteoma as a more probable diagnosis, and the clinical and pathological features of the disease supported this. After operation the patient was free of pain and remained in good health.—M. H. P.

Vascular Insufficiency of the Lower Extremity Due to Osteoma of the Femur. RANKIN, R. M. [Wash. Univ. Sch. of Med. and Barnes Hosp., St. Louis, Mo.] *Surgery*, **17**:419-421. 1945.

Case report.—W. A. B.

BLOOD VESSELS

Angiosarcomatose de Kaposi (Reticulose angioblastique). Etude hématologique et histologique. [Kaposi's Angiosarcomatosis (Angioblastic Reticulosis). Hematological and Histological Study.] RIMBAUD P., GUIBERT, H. and CAZAL, P. [Montpellier, France] *Bull. Assoc. franç. p. l'étude du cancer*, **31**:38-46. 1943.

In a case of Kaposi's angiosarcomatosis, two groups of elements have been studied: (1) the histological aspects of the lesion with demonstration of the reticulo-histiocytic origin of the neoplasms, and (2) the abnormal cells in the blood. The basic lesion is the proliferation of the reticular and fusiform cells derived from the histiocytes of the connective tissue. Kaposi's disease is more of a reticulosis than an angiomatosis.—R. J.

Sarcome périthélial de l'avant-bras. [Perithelial Sarcoma of the Fore-Arm.] MARTIN, J.-F., GUICHARD, A., and VIGNON, G. [Lab. of Path. Anatomy, Faculty of Med., Lyon, France] *Bull. Assoc. franç. p l'étude du cancer*, **32**:20-25. 1944.

Report of a case with detailed histologic description of the tumor.—C. A.

Hemangio-Endothelioma of the Diaphragm. Report of a Fatal Case in an Infant. VANALSTYNE, W. K. [Binghamton City Hosp., Binghamton, N. Y.] *Am. J. Roentgenol.*, **53**:373-375. 1945.

A case report with radiographs is presented of this rare tumor.—E. H. Q.

Treatment of Hemangiomas with Roentgen Rays. PROUTY, J. V. [Cedar Rapids, Iowa] *Am. J. Roentgenol.*, **54**:172-178. 1945.

Five types of hemangiomas are considered. *Hemangioma simplex*, simple blotchy areas on the neck in newborn infants, usually disappears without treatment. *Capillary hemangioma*, type 1, a bright red, non-elevated nevus, usually responds to treatment. Type 2, port wine stain, responds poorly to any kind of therapy. It should not be irradiated unless this can be done within the first month of life. *Hypertrophic hemangioma*, strawberry birthmark, responds well to radiation. *Cavernous hemangioma*, a soft, bulging mass, responds to irradiation unless associated with hyperplasia of fatty or fibrous tissues. In these cases the color disappears but part of the mass remains. *Spider nevi*, a network of vessels, are better treated by the electric needle than by irradiation.

Unfiltered low voltage x-rays should be used. The total dose depends on the depth and extent of the lesion, and varies from 300-600 r at a single treatment. This dose is repeated in a month and is in some cases followed by a third exposure.—E. H. Q.

HODGKIN'S DISEASE

Hodgkin's disease of the skeleton. KOOREMAN, P. J., and HAEX, A. J. Ch. [Academical Hosp., Leyden, Netherlands] *Acta med. Scandinav.*, **115**:177-196. 1943.

Five cases of Hodgkin's disease of the skeleton are presented with roentgenograms, photographs, and photomicrographs. In 2 of the patients the disease was recognized clinically. In the other 3, only osseous changes were found and histologic examination was necessary to establish the correct diagnosis. One patient had subjective, but not objective, improvement after irradiation; 3 others died after failing to benefit from irradiation, and the fifth was under treatment for too short a time for conclusions to be drawn at the time of writing.—M. H. P.

Hodgkin's Disease. Report of a Case of the Mediastinal Type with Leukopenia and Terminal Atelectasis. LEVY, S. [Wesson Memorial Hosp., Springfield, Mass.] *New England J. Med.*, **233**:322. 1945.

Report of a case of mediastinal Hodgkin's disease characterized by an unusual combination of prodromal signs and symptoms. Clinical course, laboratory and autopsy findings are included.—C. W.

ADRENAL

L'Hirsutisme avec virilisme par tumeur corticosurrénale. [Hirsutism and Virilism Due to Tumor of the Adrenal Cortex.] DUCUING, J., and BENAYGUES. *Bull. Assoc. franç. p. l'étude du cancer,* **29**:122-163. 1941.

A detailed report of a case of hirsutism with masculinization due to a tumor of the adrenal cortex, including clinical laboratory tests, descriptions, diagnosis, operative procedure, and findings at autopsy. The second portion of the report is a discussion of the syndrome hirsutism-virilism. A long bibliography is appended.—G. H. H.

Feminizing Tumors of the Suprarenal Cortex, with Description of a Case. ROHOLM, K. and TEILUM, G. [Rigshosp., and Univ. Inst. of Path. Anat., Copenhagen, Denmark] *Acta med. Scandinav.,* **111**:190-211. 1942.

Report of a case. A carcinoma of the adrenal cortex, which metastasized extensively, induced gynecomasty, and atrophy of the testis is discussed. There was excretion of abnormally large amounts of androgenic and especially estrogenic substances in the urine. The findings in 6 similar cases reported in the literature are summarized. —G. H. H.

PARATHYROID

A Case of Hyperparathyroidism. SCHRUMPF, A. [St. Joseph's Hosp., Porsgrunn, Norway] *Acta med. Scandinav.,* **115**:199-218. 1943.

Typical bone cysts resulted in increased serum and urinary calcium, marked polyuria, low specific gravity of the urine, reduced urea clearance, acidosis, and an abnormally high calcium-phosphorus ratio. These observations were made in a 60 year old man with a parathyroid adenoma. The tumor was successfully removed.—M. H. P.

MISCELLANEOUS

Teratoma of the Perineum in a Newborn Infant. LASH, A. F. [Michael Reese Hosp., Chicago, Ill.] *Am. J. Obst. & Gynec.,* **50**:344-346. 1945.

A case report is given in which a perineal tumor was removed from a newborn infant. The growth proved to be a sac lined with glands resembling rectal mucosa. The rest of the wall of the tumor was composed of muscle fibers and connective tissue.—A. K.

STATISTICS

A Study of Cancer Frequency in Stockholm. HAMMARSTRÖM, S. [State Inst. of Human Genet. and Race Biol., Upsala, Sweden] *Acta med. Scandinav.,* **112**:568-584. 1942.

From an analysis of mortality statistics in Stockholm and throughout Sweden, it is concluded that no actual increase in the frequency of cancer has occurred. Forty-six references.—M. H. P.

CANCER CONTROL AND PUBLIC HEALTH

Social Service and Cancer. A Study of 62 Gynecologic Patients. ABRAMS, R. D. [Mass. General Hosp., Boston, Mass.] *Am. J. Obst. & Gynec.,* **50**:571-577. 1945.

Rôle of the social worker in the treatment of the cancer patient is discussed.—A. K.

Cancer Facilities and Services; A Report from the National Advisory Cancer Council. *J. Nat. Cancer Inst.,* **6**:239-302. 1946.

The report is divided into four parts: Part I, Medical Education in Cancer; Part II, Basic Elements of a Cancer Program; Part III, Basic Information for Use in Studying and Planning State-Cancer-Control Activities, with Organization Charts as of 1945; and Part IV, Summary and Recommendations. In Part IV the following points are emphasized:

1—There is need for medical schools to give more comprehensive and better integrated courses in cancer.

2—The number of centers prepared to give postgraduate training in cancer is not believed to be adequate to fill the need for specially trained personnel in this field of medicine and efforts should be made to develop such training at additional medical centers.

3—Short courses of various types and other types of cancer-education activities for practicing physicians should be expanded.

4—The traineeships given by the National Cancer Institute have been very valuable and should be continued and expanded.

5—The basic elements of a cancer program within a state should include statistical research, educational activities for the public generally, and for physicians, nurses, and dentists, the provision of cancer-prevention clinics, tissue diagnostic services, diagnostic and treatment clinics, adequate hospital facilities, and adequate institutional facilities for care of the advanced cancer patient.

6—The co-operation of all agencies engaged in cancer-control activities is necessary in order to provide a complete cancer service in a state.

7—Greater efforts should be made in the field of cancer research and to increase the use of available knowledge in controlling this disease. In order that more adequate cancer facilities and services may be developed in the United States the committee makes the following recommendations:

a—That a conference of representatives responsible for the planning of cancer courses in the curriculum of schools of medicine be called with a view to developing improved methods of teaching cancer.

b—That the National Cancer Institute expand the work of training physicians in the diagnosis and treatment of cancer.

c—That the National Cancer Institute give aid in providing the physical facilities, scientific equipment, and, if necessary, the training of personnel in a few cancer centers strategically located from a geographical point of view and associated with 1 or more medical centers.

d—That the National Cancer Institute be prepared to assist state health departments and other agencies in the development of state cancer programs which will embody the facilities and services outlined in this report.

e—That in order to hasten the development of more effective means of preventing, diagnosing, and treating cancer, the National Cancer Institute expand its program of research including the training of research fellows and the program of grants to aid research in other institutions.

f—That close co-operation be maintained between the National Cancer Institute and voluntary agencies in the further development and conduct of cancer-control activities in the United States.

g—That the National Cancer Institute request appropriations sufficient to enable it to carry out the foregoing recommendations.—R. A. H.

Correction

Volume 6, No. 9, September, 1946. "Proceedings of the American Association for Cancer Research, Inc." VI. SELECTION AND SYNTHESIS OF ORGANIC COMPOUNDS, p. 489, line 11 from bottom, and p. 490, line 11 from top, suramine should read "auramine;" IX. SECOND SCREENING IN TUMOR-BEARING MICE, p. 490, line 8 from bottom, acetic-orcin should read "acetic-orcein."

Book Reviews

THE RESULTS OF RADIUM AND X-RAY THERAPY IN MALIGNANT DISEASE. Second Statistical Report from The Holt Radium Institute, Manchester, 1934-1938. Compiled by Ralston Paterson, Margaret Tod, and Marion Russell. Edinburgh: E. & S. Livingstone, Ltd. 1946, 147 pages.

This report from the Holt Radium Institute of Manchester, England, covers cases treated between 1934 and 1938. Part I is a general survey of the treatment of malignant disease as a whole intended primarily for the general practitioner. The second part is an analysis in which an attempt is made to evaluate different methods of treatment. The technics used are described or reference is made to publications in which they are fully described. Part III refers briefly to the first statistical report and gives a table showing the 10 year evaluation of the cases treated in 1932 and 1933.

As the title indicates the statistical material is limited to cases treated by radium or x-rays or both, but it includes a small number of cases in which surgery was also used. During this period 9,130 cases were seen. Of these 6,380 (70 per cent) were treated; 1,116 (12 per cent) were too advanced for treatment; the rest (18 per cent) were not treated for other reasons. It is interesting to note that 4,740 cases were treated mainly by radium and 1,640 mainly by x-rays. Forty-seven per cent of the cases treated were alive after 3 years and 40 per cent after five years. Omitting stage IV cases, 54 per cent of 4,658 patients were alive 5 years after treatment and 95 per cent of these were free of cancer. It is hardly necessary to add that in order to get the real significance of these figures the reader should consult the Report in which the numbers are given according to type, location and stage. Also, the statistical method used is described. A considerable proportion (24 per cent) of the patients treated had cancer of the skin; two-thirds being of the basal cell type and one-third of the squamous cell type.

In Part II an attempt has been made to assess the value of different technics. However, as stated by the authors themselves, through subdivision into many groups, the numbers become too small to yield reliable statistical data. Obviously, great caution should be exercized in drawing conclusions from the tables given.

In this part many controversial topics are discussed and the poor results of radiation therapy in certain fields are pointed out. For instance, immediate preoperative x-ray treatment of cancer of the breast was abandoned because of poor results and "the increased frequency of early metastases" in a small group of cases. "At the Holt Radium Institute it is believed that prophylactic irradiation of the pelvis after surgical removal of the uterus does not serve any useful purpose." There are many such statements. Whether they are justified or not, time only will determine. At least the authors present the issue squarely and unequivocally. It is for others to prove otherwise.

In compiling the 10 year tables the authors used a statistical method, suggested by an expert, which takes into account the high proportion of patients who died of intercurrent disease during this long interval. The method is sound and might well be adopted generally, although a change in terminology is necessary. The 10 year percentages do not represent an actual survival rate but the "rate of escaping death from cancer." The two rates are essentially the same when the interval is short—up to 5 years. For all cases of all types and stages totaling 1,918, treated in 1932 and 1933, the rates are: 3 year, 48 per cent, 5 year, 41 per cent; 10 year, 39 per cent. For cancer of the breast, the rates are: 3 year, 32 per cent; 5 year, 20 per cent; 10 year, 15 per cent.

The most striking thing in the report is perhaps the large proportion of patients treated by radium (or radon) almost exclusively. The results show what can be accomplished by this agent when used judiciously and painstakingly. However, a steady increase in the use of x-rays is apparent.

The report is concise and well written. The authors are to be congratulated for their successful efforts in presenting this valuable material in a form useful to the general practitioner as well as the cancer specialist.

G. Failla

GERMAN FOR THE SCIENTIST (Chemist and Physicist). Peter F. Wiener. With an introduction by E. N. da C. Andrade. First American Edition. Brooklyn, N. Y.: Chemical Publishing Co., Inc. 1946, 238 + xxi pages. Price $3.50.

This book is divided into four parts under the following headings: Part I. Grammatical Introduction (51 pages); Part II, Chemistry Passages; Part III, Physics Passages; Part IV, Translation of the Passages in Parts II and III. At the end a short German-English vocabulary (34 pages) is added.

In the grammatical introduction only the most important rules are given concerning the declension of nouns, articles, pronouns and adjectives, the conjugation of verbs and the construction of the sentence. In discussing the declension of nouns, for instance, only the general rules for endings of regular nouns and not those for irregular ones are given. This appears justified as the case of the noun can be recognized by that of the article, pronoun or adjective. The rules are illustrated by an easily understandable text with a translation of every word and a continuous translation of the whole text as an appendix at the end of Part I. Lists of irregular German verbs and of prepositions, and the few rules given, appear sufficient to enable the student to understand the logical relation of words in a sentence. A few remarks on cognates in German and English and on prefixes in compound verbs are very helpful for learning the meaning of words. It seems to this reviewer, however, that certain explanations of the functions of prefixes in changing the meaning of verbs may be misleading, as they are not general enough and do not cover certain cases. Thus on page 13, three different special functions of the prefix *ver-* are given. None of them covers words like *verbieten, verlegen, verzerren*. It might be more helpful to give a more general formulation and illustrate it by application to certain special cases.

The texts in Parts II and III were selected from important scientific papers in the field of chemistry and physics. They are long enough to appear interesting by their contents and are chosen so as to convey the knowledge of most of the important expressions found currently in the chemical and physical literature. The translations are deliberately separated in Part IV, to induce the student to make every effort to understand the text with the aid of the vocabulary only.

The book can be recommended to all students of science, scientists, and engineers who intend to read German scientific texts without having to devote much time to the study of German.

Zacharias Dische